Primates of the Caribbean: Using historical-era introduction of monkeys in the Lesser Antilles to understand patterns of island evolution

Thesis submitted for the degree of Doctor of Philosophy (PhD)
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I, Ben Garrod confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

“Nature does nothing in vain”

(Aristotle. Science of Nature)

“In all works on Natural History, we constantly find details of the marvellous adaptations of animals to their food, their habits, and the localities in which they are found”

(Alfred Russell Wallace, 1853. A Narrative of Travels on the Amazon and Rio Negro)

Abstract

Across the world, islands were and still are inhabited by unique species, often restricted to their own island and found nowhere else. After their ancestors managed to reach an island from a mainland population and become isolated from this mainland and its ecological restrictions, they often evolved spectacular adaptations. The more extrinsic barriers to gene flow there are and the more distant the populations, the greater the probability of a profound genetic and morphological change. Whereas many other insular mammalian taxa such as proboscideans, rodents and cervids react in readily identifiable trends, primates respond in varied and unpredictable ways. In order to better understand the underlying evolutionary principles behind island speciation, this thesis focuses on the small cercopithecine monkeys taken from western Africa to the Caribbean during the transatlantic slave trade. These *Chlorocebus* monkeys inhabit Nevis, St Kitts and Barbados but have long been assumed to originate solely from the Senegambia region. This thesis investigates the very early phase associated with island separation, using mitochondrial analysis and 3D geometric morphometric techniques to thoroughly assess whether any changes are present in these populations. An additional assessment is made of the three western African species of *Chlorocebus*, which is still largely subject to taxonomic discord. The results here show that the existing taxonomic status of African *Chlorocebus* does not fully describe the whole situation and that changes should be made to resolve this. The molecular results from this thesis show that rather than originating from one Senegambian location, Caribbean *Chlorocebus* monkeys instead originate from three different African species, across the entire western African coast, meaning their current designation as ‘African green monkeys’ is inaccurate. Additionally, morphological adaptations within these three insular populations are also already apparent, both across the three island groups and between Caribbean and African *Chlorocebus.*

Dedication

For my mum,
Thank you for everything.
This is *all* because of you.

Acknowledgements

This thesis is the end of a long and mostly fun journey. I first started thinking about it many years ago working in Africa chasing chimps and now ten years later, it represents the culmination of a lot of work, a lot of effort and a lot of sacrifice and it feels as though I'm at the end of that journey now. It's not been easy and I've very definitely realised that in trying to juggle a PhD, it's the life around it that can often be the hardest part. Since starting my thesis, I've had meningitis, broken bones, a broken heart, a kidney disease and a few other 'ailments' but whatever the obstacle, I've always had the PhD there beside me, reinforcing why it is I do what I do. I guess I owe the PhD itself a 'thank you' here for teaching me more than just about monkeys and their evolution. Throughout my work, a number of people have helped in various ways, always ready to offer their advice, expertise or sometimes just a smile. To all of them, I am deeply indebted and shall endeavour to mention their names here but for all those who helped that I don't mention, please know that I am extremely grateful.

First and foremost, I would like to express my sincerest gratitude for my supervisors Prof Helen Chatterjee and Dr Sam Turvey, without whose constant support, advise and humour I would never have gotten to this point. I feel so privileged to have been able to work as their student and benefit from their insight and expertise and believe that I could not have had two better supervisors. I know it's not always been plain sailing but your patience and support has brought me to this point. I feel lucky to end this PhD not only submitting a thesis but leaving with two very dear friends. Thank you for everything.

A very special thank you needs to go to Ian Barnes and Selina Brace (Royal Holloway, University of London and Dept. Earth Sciences, British Natural History Museum, London). Their help throughout my genetics work

was invaluable and I am deeply thankful for all the time and effort they spent with me to help achieve that. At a moment's notice, I was always able to use the aDNA labs and could always call for advice and help and for that, I owe you both.

Caribbean fieldwork featured heavily in my thesis and numerous people should be mentioned here for their help and support. In Nevis, Evelyn Henville (Nevis Historical and Conservation Society) and Emile Pemberton greatly helped me in my work and Bev Parry, Jim Johnson (RIP) and Pam Barry were all a big part of my time there. Nevis now holds a very special place in my heart and they have all helped forge this attachment. I'm sure I'll be back there soon. In Barbados, the help of Julia Horrocks (Dept. Biological and Chemical Sciences, University West Indies) was essential in securing my work with Jean Baulu and his team at the Barbados Primate Research Centre. A huge thank you goes to Genevieve Marsh, Carlisle Sutton, Linnell Banfield and the rest of the team for greatly facilitating my work there and for making me feel welcome at the centre. The support of staff at both UCL and the Zoological Society of London (ZSL) has always been of great use and I am incredibly grateful for any and all support they have given. A special thank you to Kevin Fowler, Julia Day and Manu Davies for your help with an almost unlimited number of topics – from the ordinary admin side of things and help with grants, to help with avoiding being 'stuck' in the Caribbean for too long. Also, both Amrit Dehal and Jo Keogh from ZSL have provided constant support and help at various points across the last few years.

For individual chapters, I must thank Katharine Balolia (Australian National University, Australia) and Ryan Felice (UCL) for their help with morphometrics. Never have I encountered such temperamental software but you were both there to help make sense of it all. For my pathology chapter, I must say a huge thank you to Peter Stafford and Chris Thorn who were not only both excessively enthusiastic but who provided all xray

material for free for that chapter. Thank you both of you. A huge thank you is made here to Heather Bonney (Dept. Earth Sciences, British Natural History Museum, London) who was literally invaluable in her help with the CT analysis of monkey skulls. Working with Heather is an example of not only how this PhD has helped develop strong research connections for future collaborations but strong friendships also.

The use of museum material in this thesis was central to the research and several collections featured heavily. Roberto Portela Miguez, Richard Sabin, Louise Tomsett and Paula Jenkins all from the Mammal Department (British Natural History Museum, London) were continuously a source of help and support. There was a lot of scanning and it seemed to take forever but they were brilliant throughout. The collection at the Royal College of Surgeons, London was of huge importance and I very definitely found my feet in terms of 3D scanning there. The help of Milly Farrell, Sam Alberti, Carina Phillips and Martin Cooke made this very difficult and long part of my PhD that much easier and I owe them all a debt of gratitude here. Curatorial staff from other collections across the UK deserve a mention here for their help, even if only one skull was available or indeed it was actually from the wrong species. Thank you Jack Ashby (Grant Museum of Zoology and Comparative Anatomy, London), Mark Carnall (Oxford University Museum of Natural History, Oxford), Isla Gladstone (Bristol Museum and Art Gallery), Paolo Viscardi (Grant Museum of Zoology and Comparative Anatomy, London) and the role of the Natural Sciences and Collections Association (NatSCA) for their help when needed. My work through this thesis has led me to create a much stronger working relationship with UK museums and I hope to continue this long into the future.

Various friends, family and colleagues have been there for me throughout my PhD with as many different ways of supporting me as I can number and I am hugely grateful to each and every one of them but a few deserve

special mention here. Ross Barnett and Rhys Jones were brilliant when I needed to bounce ideas back and forth and were kind enough to read through various chapter drafts and provide useful feedback. Toby Carter, Phillip Pugh and Andrew Smith were always there to help guide me through answers to questions, even if I didn't know I was asking them. After more than a decade, I am still learning a great deal from each of them and owe them my thanks here. John Hutchinson and Alice Roberts have always both been just at the end of the phone if and when they've been needed and their help and more importantly their role as friends has been of the greatest support. I need to thank Jane Goodall for not only helping me decide to embark on a PhD long ago in Africa but in her help and words of wisdom throughout the writing of this thesis and for being a point of strength when I've needed it most and to David Attenborough for taking a keen interest in my morphometrics and for running me through my paces well before I wanted to be examined on it. His words of support and help in making me see the bigger picture when I really needed it the most helped get me back on track right towards the end of the thesis. A huge thank you must go to my dearest friend Claire Thompson, closest pal Lauren Pascoe, best mate Bonnie Griffin and long-suffering ex-housemate Lydia Baines. You've all been the endless supply of wit and humour, questions and ideas and cups of tea throughout. Between the four of you, you've shared in every single part of the PhD – all of it! Thank you guys, it's meant a huge amount to me.

I need to dedicate a special thanks to Stephanie Campbell for her love, support and help. She has been right beside me, day and night and has helped give me the determination to get to this point now. I would like to thank her for everything and for not giving up.

Lastly, and most importantly, I must thank my family. I could not ask for a better mum, dad and brother (even if he still doesn't really know what it is that I do) and I definitely would not be where I am today without their

unending love, support and help. Long ago, mum said that the road in life is not a straight path and that there are many little lanes off to the side that I should explore. Thank you so much you three for always encouraging me to explore these little side lanes. Everything I achieve in my life will ultimately be down to you. Thank you for always being there, every single step of the way.

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Chapter 1

General Introduction

1.1 Introduction

Island environments typically differ from comparable mainland habitats in numerous respects. Those populations introduced onto smaller islands for example very often have fewer resources, may experience a reduction or absence in predation and may face less interspecific competition. Because of such changes, introduced insular populations are often subject to drastically increased rates of evolutionary change (on both a genotypic and phenotypic level) and subsequently to rapid speciation (Schillaci *et al.* 2009). Distinct adaptive differences are likely to arise within a population if that population is reproductively isolated for a substantial period of time (Okada *et al*. 2014). Such reproductive isolation allows for the rapid acceleration in a population's divergence due to increased genetic drift. This increase may then lead to founder effect events in populations where genetic variation is limited (Okada *et al*. 2014). Species that are introduced to island ecosystems are often subject to a general trend in morphological change. Smaller species may show adaptations leading to gigantism, whereas dwarfism is seen in larger introduced species. This phenomenon is widely known as the 'island rule' (Van Valen, 1973). Different taxa typically respond to island separation in predictable ways, with carnivores, artiodactyls and heteromyid rodents becoming smaller in insular ecosystems and murid rodents becoming larger (Meiri *et al*. 2008). However, the applicability of the island rule in primates is still largely unresolved, in part due to conflicting findings. The results from one study (Bromham and Cardillo, 2007) found that the island rule does have an effect across multiple primate species,

with insular populations of Japanese macaques (*Macaca fuscata*), pig-tailed langurs (*Simias concolor*) and purple-faced langurs (*Trachypithecus vetulus*) being 80, 52 and 61% (respectively) the size of their mainland counterparts. The results from other studies however do not support a correlation between insular primate populations and changes in size (Meiri *et al*. 2008; Schillaci *et al*. 2009).

This thesis sets out to look at possible genotypic (mitochondrial) and phenotypic (cranial morphometric) differences in three populations of African green monkeys (*Chlorocebus* spp.) that were taken from Africa to the Caribbean several hundred years ago as part of the Transatlantic slave trade. Within this research, a range of methods were used to investigate any variations within these introduced cercopithecine primates; including the use of ancient DNA (aDNA) to assess the mitochondrial (mtDNA) phylogeny of both the species in Africa and the introduced Caribbean populations and 3D geometric morphometrics of the skull. Additionally, an assessment was made of an observed population-level cranial deformity found within the Caribbean-living monkeys. The data from this research may not only resolve if and how introduced primates are subject to insular effects but on a wider scale may also help elucidate whether intra-island speciation has been an important factor in generating species diversity in the Caribbean mammal fauna, as this is still largely unresolved (Brace *et al.* 2012).

1.2 Thesis objectives

Within the Caribbean, the introduced medium-sized *Chlorocebus* monkeys represent an important area for studying island biogeography. Understanding how these monkeys became established within the Caribbean may not only aid our understanding of the processes involved with insular colonisation but can also reveal how a multidisciplinary approach may be used to understand such events. Additionally, Caribbean *Chlorocebus* monkeys are extensively used in biomedical (AIDS) research, to investigate viral replication and immune responses in natural hosts infected with SIV (Pandrea *et al*. 2006), with many procedures based on the assumption that they originated from the Sene-Gambia region of Africa.

Previously, 17 mitochondrial DNA samples from two Caribbean populations (Barbados and St Kitts) were compared with 14 individuals from three *Chlorocebus* species in Africa (from the Central African Republic, Tanzania and Senegal). Importantly, the possibility that genetic lineages represented by founder monkeys imported from other parts of Africa may have been lost, partly due to inbreeding (Van der Kuyl *et al*. 1996) was proposed.

Chapter 2 sets out to elucidate the largely unresolved phylogeny of the African *Chlorocebus* clade and investigates if and how the introduced Caribbean Chlorocebus fits within this phylogeny. Using mitochondrial DNA and using ancient DNA methods, this chapter investigates whether (1) the Caribbean

populations of *Chlorocebus* represent a single or multiple colonisation event, (2) if the Caribbean *Chlorocebus* monkeys originate from a single or multiple African source population, and (3) whether the phylogeny supports the current species designations in the African *Chlorocebus* species.

Chapter 3 uses *Chlorocebus* morphometrics including the basicranium, maxillofacial and temporal region to provide a quantitative analysis of cranial shape among the African *Chlorocebus* taxon to 1) identify major patterns in cranial shape across the group; 2) identify whether any cranial morphometric divisions correspond with existing current *Chlorocebus* taxonomy and; 3) see whether the findings help resolve the *Chlorocebus* taxonomic ambiguity. An assessment is also made of the three populations of Caribbean *Chlorocebus* monkeys, using morphometrics to see whether there are quantifiable differences 4) between these three insular populations and; 5) if there is any correlation between these Caribbean monkeys and any of the African *Chlorocebus* taxa. This study focuses on shape variation and differences across the crania.

Although it is not uncommon to encounter skeletal pathologies in captive primates, examples of such non-injurious pathologies at a population level in wild-living primates are much rarer. *Chapter 4* sets out to describe and investigate the gross pathological findings and aetiology associated with a previously undescribed, population-level cranial pathology seen in the wild-living Caribbean populations of *Chlorocebus* monkeys and to investigate whether any causative factors can be associated with this pathology.

The main findings are integrated and summarised in *Chapter 5*, along with a discussion on the likely phylogeographic history or histories of the introduced Caribbean *Chlorocebus* monkeys, how their island isolation may have influenced them and whether this fits a pattern observed in similar species. The results are then put in a broader context and their implications for the understanding of reactions in primate species to insular isolation are discussed.

1.3 Biogeography in African cercopithecine primates

Across much of Africa, Plio-Pleistocene climate change (starting approximately 5.3 Ma) caused dramatic shifts in faunal assemblages (Bobe and Behrensmeyer, 2004; deMenocal, 2004) a combination of lower temperatures coupled with increased aridity in East Africa reduced and fragmented tropical forests, leaving them as isolated patches along major rivers and areas of high elevation (Bobe and Behrensmeyer, 2004). This creation of a patchwork of forested habitats has contributed in part to the rapid evolution and diversity of the African cercopithecine (tribe: Cercopithecini) and continues to do so today (Kamilar *et al*. 2009). Primate populations that have experienced recent habitat loss and fragmentation show low levels of genetic diversity, due to elevated genetic drift (Mbora and McPeek, 2010) and in those species characterised by female philopatry, a mtDNA pattern which is relatively homogenised within populations, as is found in forest patches for example, but heterogeneous between populations (Shimada, 2000; Mbora and McPeek, 2010) is typically seen. These discrete habitats act as a mechanism of geographic isolation and it is probable that recent phylogeographic history has shaped the pattern of genetic differentiation (Carden *et al*. 2012) in many of the African monkeys. Although there is often a level of discordance between mitochondrial phylogeny and morphology, many of the African non-human primate taxa such as the guenons and papionins can be broken down into several well-supported major haplogroups, reflecting distinct geographic populations (Zinner *et al*. 2009). The cercopithecines are a species-rich group of African monkeys that provide an

ideal model for investigating areas such as speciation and the processes involved in colonisation within evolutionary biology (Kamilar *et al*. 2009). The group includes subsets of taxa where some species and populations are characterized by having overlapping ranges, whilst others are geographically separated, making detailed comparisons possible. Additionally, range maps are known for the vast majority of cercopithecines and because some species are widely used in biomedical research, their molecular profiles are well studied. There is however very often a sizeable level of discord between morphological and molecular traits in terms of establishing taxonomic status when looking at either mtDNA and/or nuclear DNA (nDNA) in the wider group of cercopithecines (Detwiler *et al*. 2005; Zinner *et al*. 2011). Successful captive matings and the occasional reports of hybridization between different cercopithecine taxa show that many species are at least capable of reproducing but because such pairings are a relatively rare occurrence in the wild, it appears that this behaviour is selected against (Allen *et al.* 2014) and that cercopithecine facial patterns have evolved under selection to become more visually distinct from those of other species with whom they are sympatric (Allen *et al.* 2014). The identification and distinction of conspecifics from other closely related species appears to have been a major component of diversification and speciation for these primates, acting as isolating barriers.

1.4 *Chlorocebus* monkeys

Chlorocebus monkeys (often generically referred to as green monkeys or vervets) are the most widespread of the African monkeys and inhabit large parts of sub-Saharan Africa. These haplorrhine monkeys (superfamily: Cercopithecoidea) are typically around 420 - 600 mm in length with a tail adding an extra 300 - 500 mm to the total length. Body mass typically ranges between 3.0 - 8.0 kg, with an average female weight of 4.1 kg and male weight of 5.5 kg in these highly sexually dimorphic primates (Kingdon, 1997; Groves, 2001). Typically, these monkeys have relatively short hair covering most of their body, have black or dark faces and elongated pale side whiskers bordering much of the face (Napier, 1981; Groves, 2001). A defining feature of the group is the green coloration around the face, created by the banding of yellow and black-stranded hairs.

Chlorocebus monkeys are highly social and one of the few taxa to have multi-male groups. Rank dominance amongst males is demonstrated by placing the tail in an erect vertical position as they pass other group members. They are highly omnivorous, eating a range of fruits, insects, vegetation, and even small mammals and birds, using the cheek pouches characteristic of the family. They are semi-terrestrial and semi-arboreal, often spending the daytime on the ground and retreating to trees at night (Fedigan and Fedigan, 1988).

1.4.1 African *Chlorocebus* monkeys

The African *Chlorocebus* group (known also as 'savanna' monkeys) are medium-bodied primates, splitting their time between terrestrial and arboreal foraging (Groves, 2005; Kingdon, 2015) in environments characterised by continuous belts of (deciduous) vegetation that often follow drainage lines. These monkeys forage on the ground some appreciable distance from the trees on which they rely for food and shelter. Depending on resource availability, African *Chlorocebus* species form either large groups which are able to utilise and defend resource abundant, small (as little as 13ha) territories or smaller groups which disperse across more resource-poor, larger (up to 178ha) territories that cannot be exclusively defended (Kingdon, 2015). Typically, *Chlorocebus* monkeys are broadly omnivorous, with seeds, flowers, foliage and small animal foods all forming part of their diet. Fruits however, are their preferred and most important dietary component, with the seeds, leaves and gum from *Acacia* being an essential component (Groves, 2005). A high level of fecundity allows the group to recover after any major population drops resulting from severe fluctuations in food sources and to offset the heavy level of predation caused by predatory raptors, felids and reptiles (Kingdon, 2015). The taxonomic status and even the exact number of species of African *Chlorocebus* monkeys is controversial, which has been reflected in the conflicting and confusing classification of the group (Napier, 1981). In addition to the fact that none of the taxa are actually green in their appearance, it is thought that this long history of taxonomic uncertainty arose mainly due to a failure to

study living animals, since museum specimens are often lacking the areas of their anatomy – head, feet and tailtip – traditionally necessary to make a proper identification. Recent molecular work focusing on the mitochondrial diversity of African *Chlorocebus* monkeys (Haus *et al*. 2013) showed that mtDNA diversity does not conform to the existing taxonomic classification of the group and that in several instances, there were clear examples of disparity between phenotypes and mtDNA status, possibly relating to probable contact zone hybridisations. The African *Chlorocebus* taxa were previously subsumed into the *aethiops* species group of the *Cercopithecus* genus (Schwarz, 1926; Dandelot, 1959; Napier, 1981; Groves, 2001) at a subspecies level, but in 1907 Pocock was the first to elevate one of these subspecies to the specific rank, taking the green monkey from *Cercopithecus aethiops sabaeus* to *Cercopithecus sabaeus*. Subsequently, *C. sabaeus*, *C. aethiops* (consisting of *C. aethiops aethiops* and *C. aethiops tantalus*) and *C. pygerythrus* (comprising *C. pygerythrus pygerythrus* and *C. pygerythrus cynosuros*) were separated (Dandelot, 1959), with both *tantalus* and *cynosuros* being elevated to the specific level at a later date (Dandelot, 1971). Subsequently, the name *djamdjamensis*, which was previously a synonym of *aethiops*, was resurrected to represent a possible distinction within the taxa (Dandelot and Prévost, 1972) and was later raised to the specific level (Kingdon, 1997). Throughout the duration of this period, the taxon has been the subject of much and often heated debate, ranging from a single species as proposed by Schwarz (1926), to that of Thorington and Groves (1970), where 22 subspecies subsumed into four subspecies groups were identified (Napier, 1981), which were broken down into 'northern' (*sabaeus*,

tantalus and *aethiops*) and 'southern' (*pygerythrus*) groups. More recently, six species were taken from *Cercopithecus* and placed into their own genus *Chlorocebus* (Table 1.1) as a sister taxon to other ground-dwelling semi-arboreal members of the Cercopithecini, such as *Erythrocebus* and *Allochrocebus* (Haus *et al*. 2013). There is currently still much debate with regards to the classification of the *Chlorocebus* group: namely, whether they are one polytypic species (*Chlorocebus aethiops*) and are subdivided into several subspecies (Kingdon, 1997; Elton *et al*. 2010), or whether they form six distinct species (Groves, 2001); green monkey (*Chlorocebus sabaeus*), tantalus monkey, (*C. tantalus*), malbrouk monkey, (*C. cynosuros*), vervet monkey, (*C. pygerythrus*), grivet monkey (*C. aethiops*) and the Bale monkey (*C. djamdjamensis*), with both *C. tantalus* and *C. pygerythrus* being polytypic (Fig 1.1). Confusingly, the whole taxon is still widely termed as vervet monkeys (Kingdon, 1997; Cardini and Elton, 2008: Elton *et al*. 2010). Within this research, the taxon is referred to as '*Chlorocebus* monkeys' and the taxonomy of Groves 2001 is followed, accepting six distinct African species; *C. sabaeus, C. tantalus, C. cynosuros, C. aethiops, C. pygerythrus,* and *C. djamdjamensis.*

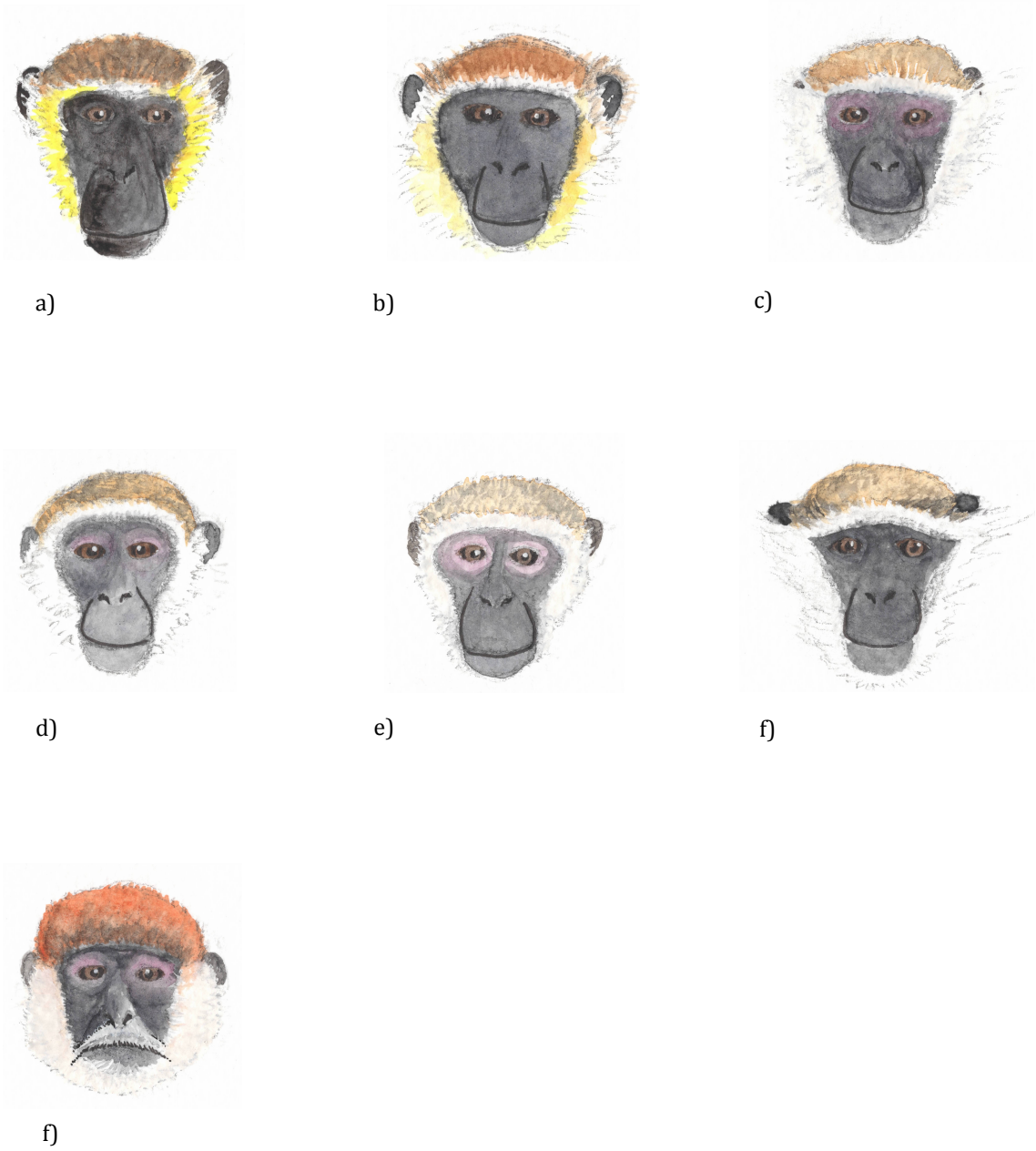

Figure 1.1 Showing the main facial characteristics for the six described African species of *Chlorocebus* and a representative Caribbean *Chlorocebus* face. All images are based on adult male faces. Images are redrawn from Kingdon (1997) and Hill (1966), with a) Caribbean *Chlorocebus*, b) *C. sabaeus,* c) *C. tantalus,* d) *C. cynosuros,* e) *C. aethiops,* f) *C. pygerythrus,* and g) *C. djamdjamensis.* Redrawn from Kingdon, 2015.

Table 1.1. Details on the main phenotypic characteristics for the six African *Chlorocebus* species (Napier, 1981; Groves, 2001).

	Geographic range	**Brow band**	**Whiskers**	**Face**	**Colour at base of tail**	**Hands and feet**	**Tailtip**
C. sabaeus	Senegal, Gambia, Sierra Leone, Liberia, Cote d'Ivoire, Burkina Faso, Ghana	Absent	Yellowish, forward facing forming a crest: sharp contrast with crown.	Black.	Scrotum pale blue.	Light-coloured - never black.	Golden yellow.
C. tantalus	Togo, Benin, Nigeria, Cameroon, Chad, CAR, S. Sudan, DRC, Uganda.	Narrow – separated from whiskers by line of black hair around eyes.	Long, stiff, yellowish, sometimes speckled at tips – in contrast with crown.	Black.	Tufts of white & orange hair around tail base. Scrotum sky blue.	Light-coloured - never black.	Whitish.
C. aethiops	S. Sudan, Sudan, Ethiopia.	Narrow – confluent with whiskers.	Long, white, softly curved – in contrast with crown.	Black with white moust-ache	Tufts of white hair around tail base. Scrotum sky blue.	Light-coloured - never black.	Whitish.
C. pygerythrus	Ethiopia, Somalia, Kenya, Uganda, Tanzania, S. Africa, Zimbabwe, Mozamb.	Broad – confluent with whiskers.	Short, white – blending with crown.	Black.	Tufts of red hair around tail base. Scrotum turquoise blue.	Black or dark.	Black or dark – on dorsal aspect.
C. cynosuros	Gabon, Congo, DRC, Angola, Zambia, Botswana	Tapered white	Long, stiff, yellowish – with short black tips.	Variable – from black to flesh-coloured	Tufts of red hair around tail base. Scrotum lapis lazuli blue.	Pale	Creamy white.
C. djamdjamensis	Ethiopia	Barely visible white.	Short, bushy white beard,	White moust-ache	Inconspic uous red tufts. Scrotum sky blue.	Dark grey.	Reduced or virtually absent.

Some primate genera have a complicated taxonomic history, giving rise to disagreement over numbers of valid taxa (Mercês *et al*. 2015) and the African

Chlorocebus monkeys fall well within this category. They represent a widely distributed and morphologically diverse sub-Saharan genus, with the six presently recognised species being separated largely on the basis of morphological characteristics and not on molecular differences (Haus *et al*. 2013) and are still the subject of broad taxonomic discordance. Despite mitochondrial analyses, and specifically cytochrome *b* (Cyt *b*), being used to distinguish closely-related species of primates (Mercês *et al*. 2015), based on a mitochondrial phylogeny, there is a discrepancy between the number of *Chlorocebus* species recognised through their morphology and the number of clades stemming from molecular results (Haus *et al*. 2013; Mercês *et al*. 2015). In looking at possible morphological differences within the African *Chlorocebus* genus across the described species, distinct variation in cranial morphology in African skulls has been observed, showing an association with a strong environmental and spatial basis (Cardini *et al.* 2007), where such variation was associated with longitudinal rather than latitudinal differences.

1.4.2 Introduced Caribbean *Chlorocebus* monkeys

Before 1866, the Caribbean monkeys were neither specifically named within reports nor were they unambiguously described. Although official government bounty laws date back to 1682, these documents only refer to 'monkeys' in general and do not attribute a species to these animals. It was only in 1911 that they were confirmed as being *Cercopithecus aethiops sabaeus*, in line with the taxonomic consensus at the time (Denham, 1981). From their first mention, African-originating Caribbean monkeys were said to have been sourced from the Guinea Coast region and whilst there was said to be a remote possibility that the monkeys were from elsewhere in Africa, this was ruled as being highly improbable (McGuire, 1974). Before this 1911 identification, Schomburgk in 1848 had identified the early feral population of monkeys on Barbados as *Cebus*. There is some evidence that capuchins were already on the island when *Chlorocebus* monkeys were introduced but due to a paucity in records which accurately describe the monkeys prior to the 19th century, it is unclear which species was there in the mid 17th century and when exactly *Chlorocebus* monkeys became established there (Denham, 1981). On each of the Caribbean islands inhabited by *Chlorocebus* monkeys, a large degree of phenotypic variation is often apparent between populations (Sade and Hildreth, 1965; Poirier, 1972). In addition to the large amount of colour variation seen within the St Kitts population for example, the Caribbean *Chlorocebus* monkeys typically have faces margined with bold yellow hair rather than the white facial hair seen in the majority of the African *Chlorocebus* monkeys and much brighter than the

yellow facial hair seen in the African *C. sabaeus* (Sade and Hildreth, 1965; Denham, 1981). Across the Caribbean populations, observable differences in terms of patterning are also seen (Poirier, 1972). Additionally, parasitological studies have shown that the Caribbean monkeys possess a species of intestinal nematode not seen elsewhere (Denham, 1981).

In early morphometric analysis, 68 cranial dimensions were measured, showing that Caribbean skulls (at least those from St Kitts) were both bigger and less variable than those of African *Chlorocebus* (Ashton and Zuckerman, 1950; Ashton *et al*. 1978). Whilst no changes in meristic, quantitative cranial characters were identified, significant divergence was seen in the increased size of the Caribbean skulls, especially in terms of breadth (Ashton and Zuckerman, 1950). The changes in skull morphology that were observed reflect changes seen in Caribbean *Chlorocebus* dentary (Ashton and Zuckerman, 1950; Ashton *et al*. 1978). When looking at the cranial dimensions, St Kitts monkeys for example tend to be bigger and less variable than in the African monkeys (Ashton and Zuckerman, 1950; Poirier, 1972) and show a greater frequency of dental abnormalities of an 'all or nothing' nature, such as the addition or subtraction of a tooth (Ashton and Zuckerman, 1950; Sade and Hildreth, 1965). Variation in the normal number of tooth roots on the third molar was found in over 20% of St Kitts monkeys, which is significantly more than that found in African *Chlorocebus* monkeys. In approximately 25% of the St Kitts skulls, one or both of the first incisors protruded beyond the line of the second incisors (Sade and Hildreth, 1965). Recent molecular results show that the Caribbean

Chlorocebus populations are phylogenetically clustered, appearing to represent populations that stem from a single source population (Brown *et al*. 2013). Using cytochrome *b* sequencing, these Caribbean monkeys have been classified as being *Chlorocebus sabaeus* and most likely originated from the Sene-Gambia region (Van der Kuyl *et al*. 1996; Pandrea *et al*. 2006).

Currently, Caribbean *Chlorocebus* monkeys are somewhat confusingly classified as vervets on one island at the same time as being classified as green monkeys on another (Horrocks, 1982; Horrocks and Hunte, 1983) but it is widely believed that as a whole, the Caribbean population is a population of green monkeys (*C. sabaeus*), originating from western Africa from the region around Senegal. The presence of individuals with characters intermediate between two or more closely related species causes problems in the identification of taxa, especially when genetic markers identify alternative species boundaries. This can further lead to false conclusions about allopatric speciation, particularly for island populations where differentiation at the specific level may be subjective. The intermediates may be either hybrids between two species or reflect natural variation within a single species (Gillespie *et al.* 2013). With such an unclear taxonomic status, it is possible that the Caribbean *Chlorocebus* monkeys are either showing evidence of early speciation that has begun as a result of an initial founder effect combined with three centuries of genetic drift or natural selection or they are displaying signs of a complex history of migration that has yielded monkeys of mixed parentage. Despite what is widely accepted regarding the Caribbean monkeys, Denham (1981) seems alone in identifying that the

Caribbean population may have had ‘innumerable sources of origin’ anywhere south of the Sahara from anywhere along the coast.

1.4.3 The introduction of *Chlorocebus* monkeys to the Caribbean

It is widely believed and accepted that the Caribbean *Chlorocebus* monkeys were transported from Africa to the Caribbean between the 17-19th centuries. Whilst there is uncertainty around when monkeys were first introduced, from records it is evident that they had become well established by the late 1600s, most likely resulting from multiple introductions occurring between 1627-1807 (Denham, 1987). Although it is unknown how and why monkeys were first introduced to Barbados, the St Kitts introduction is a little clearer. Settled by Europeans in 1624, first of all by the English and then by the French shortly after, the first century of European occupation was marked by continuous conflict between the two, before being ceded entirely to the British in 1713 (Sade and Hildreth, 1965). It is believed that it was during such skirmishes that captive monkeys escaped from the homes of French settlers. The Barbados population is thought to have also started from such accidental releases. The Nevis population is thought to have been deliberately introduced in the mid 17th century, despite the fact they were already considered to be pests on St Kitts and Barbados (McGuire, 1974). While it is unclear exactly when feral breeding populations originated in the Caribbean, the St Kitts population was already subject to a bounty by 1682, as they represented a serious agricultural threat through crop-raiding (McGuire, 1974; Denham, 1987).

In addition to enslaved Africans, ships travelling from Africa to the Caribbean routinely transported gold, ivory, wax, skins and gums, pepper, herbs, fabrics,

parrots and monkeys (Poirier, 1972; Eltis and Richardson, 2010). Whilst it is unclear as to whether the monkeys were taken to be sold as pets or for the purposes of eating, it does seems likely that the pet trade would have largely fuelled their transportation (Sade and Hildreth, 1965). In one instance from the same period, one report documents that over 330 monkeys were taken from Africa to France for the pet trade on a single ship and that such occurrences were not rare. Whilst this example does not involve the Caribbean, similar numbers may have been transported across the Atlantic, meaning that early Caribbean monkey populations were much higher than might be expected (Denham, 1981). Early records state that monkeys were widely kept as pets on St Kitts as a high status commodity until the early 17th century, by which time they had widely become feral (Denham, 1981). Taking into consideration that both green monkeys (*Chlorocebus sabaeus*) and tantalus monkeys (*C. tantalus*) were popular in 17th and 18th century menageries in Europe (Denham, 1981) and that there are accounts of them being highly prized as pets in the Caribbean, it is likely that even if they were introduced at a rate of one animal per 20 ships, the introduced population would have been at the very least in the hundreds (McGuire, 1974; Denham, 1981). Within the Caribbean, *Chlorocebus* monkeys are collectively known as 'African green monkeys' and from historical accounts and records such as early colonial reports and slave ship log books, they have long been considered to be coastal West African in their origin, being transported in their hundreds from multiple ports from around Senegal, Gambia, Gabon and Nigeria (Denham, 1987; McGuire, 1974). The earliest account of the origin of the introduced Caribbean *Chlorocebus* monkeys was mentioned in a

1722 report by French missionary Labat, stating that slave ships arrived into the Caribbean from the Sene-Gambia region, which largely reflects the French slave trade from French-controlled African countries to Franco-Caribbean islands (Eltis and Richardson, 2010). However, when combining the French, British and Dutch trade in enslaved Africans, less than 10% of slaves arriving in the Lesser Antilles originated from the Sene-Gambia region (Denham, 1981; Eltis and Richardson, 2010), with the remainder being taken from the whole length of the western coast and parts of the eastern region (Fig. 1.2).

Enslaved Africans were transported to the Caribbean by the million, with the Caribbean and South America accounting for more than 95% of the demand for the slave trade. Throughout the years that the trade was active (between 1501-1867), the points of embarkation in Africa that would subsequently lead to disembarkation in the Lesser Antilles changed (Fig. 1.3). Over time, the trade moved further south, changing from a focus around Senegal and Gambia, on to the region around Nigeria and eventually progressing right down to Angola, eventually encompassing almost the entire West African coast (Eltis and Richardson, 2010). Between 1501-1641, the majority of enslaved Africans were taken from Senegambia right down to the West Central Africa (WCA) region, in a trade dominated by the Spanish and Portuguese. In the period 1642-1807, over 75% of the trade was controlled by the British and Portuguese, who focused trade between the Windward Coast and WCA and as the legal trade ended in 1807, the subsequent trade from 1808-1867 focused between the

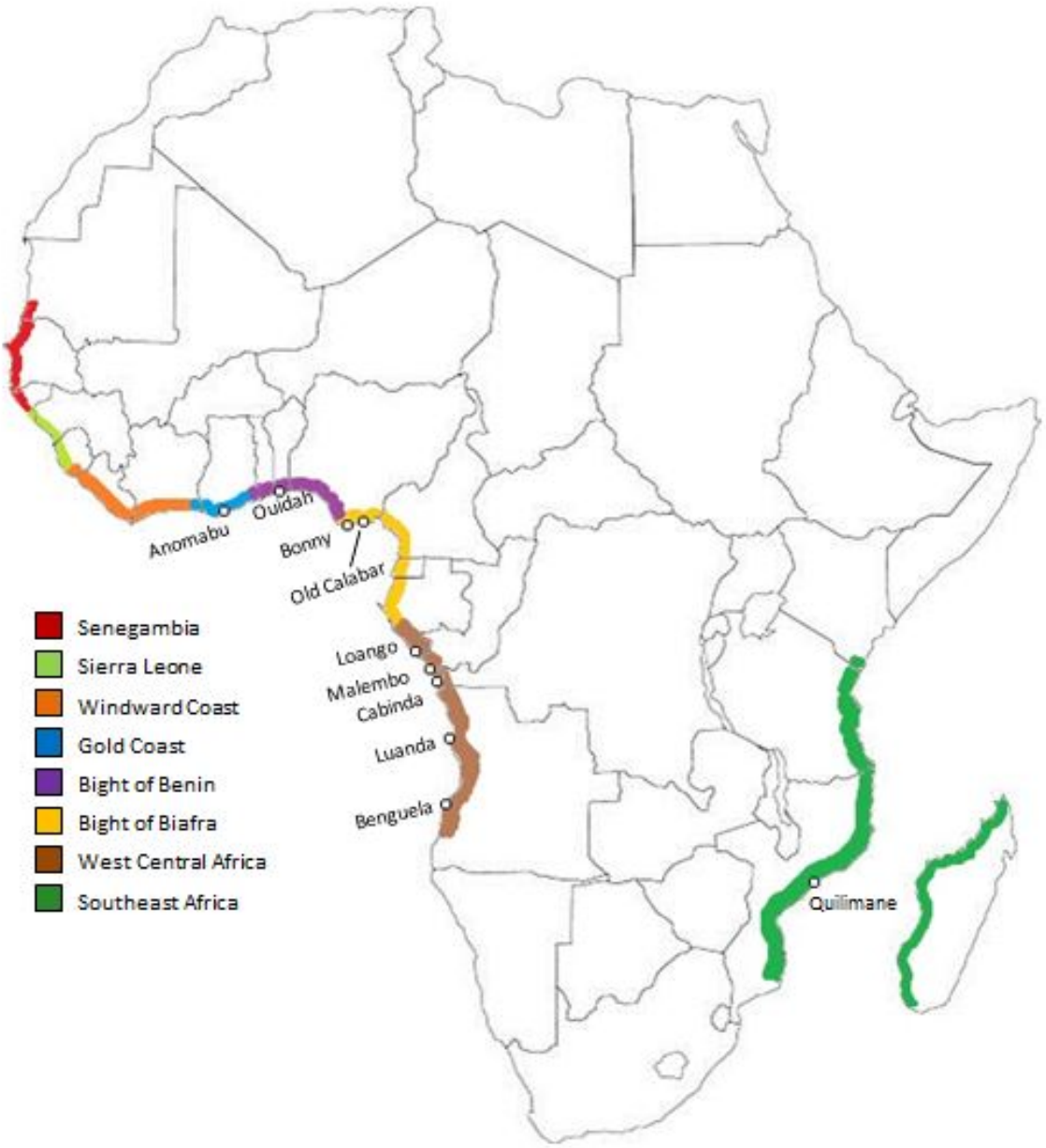

Figure 1.2. The transatlantic slave trade exploited eight distinct regions along both the western and eastern coast of Africa. Whilst there were other points of embarkation, these eight regions (with ten major ports) accounted for more than 10,700,000 of the enslaved Africans taken to the Americas. Data taken from Eltis and Richardson, 2010.

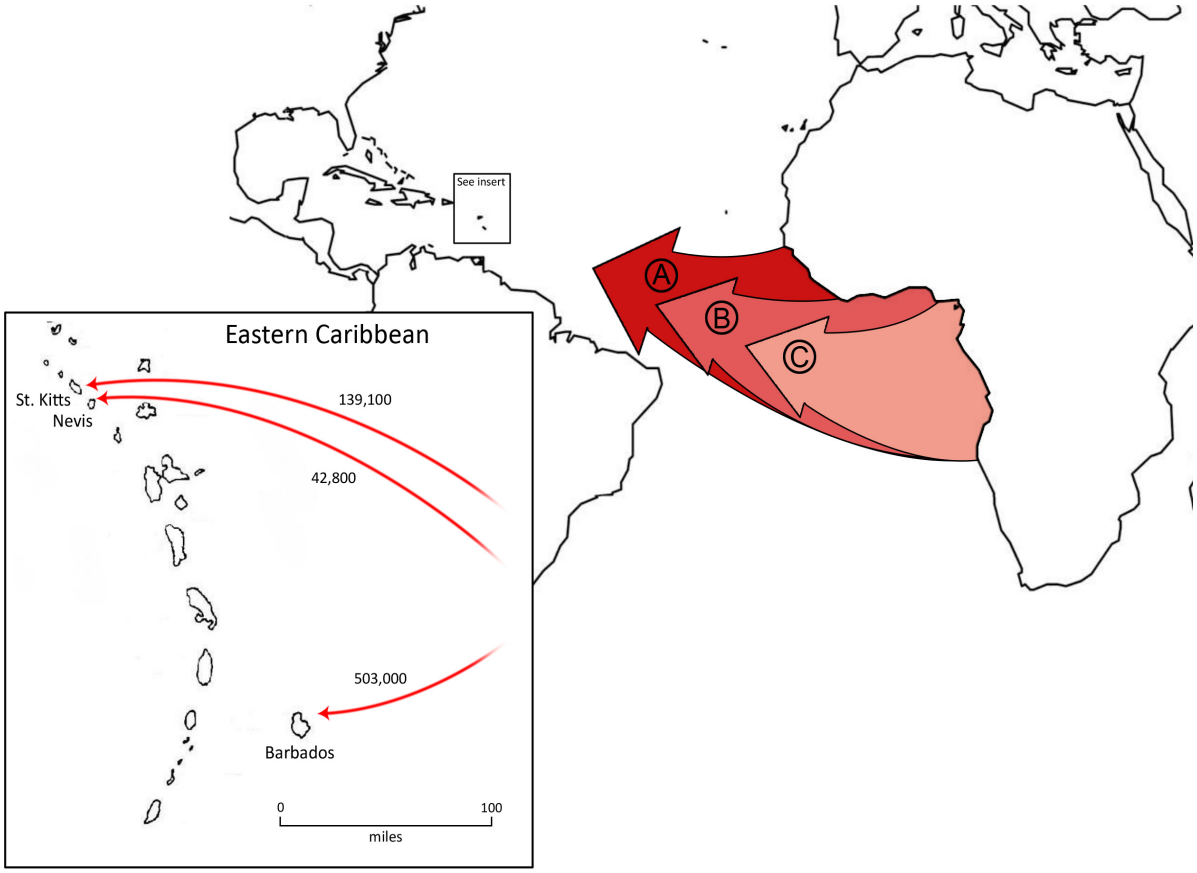

Figure 1.3. Across the three centuries of the transatlantic slave trade, the focus of the trade shifted from Senegambia to West Central Africa (WCA) between 1501-1641 (A), then with a focus between the Windward Coast and WCA between 1642-1807 (B) and finally, after the trade was made illegal, the focus narrowed to the region between the Bight of Biafra and WCA (C) between 1808-1867. Data taken from Eltis and Richardson, 2010.

Bight of Biafra and WCA (Table 1.2). Throughout the trade to the Caribbean, French vessels predominantly supplied the Greater Antilles, whilst the Lesser Antilles (covering the three islands where *Chlorocebus* monkeys would

subsequently become established) was largely supplied by the British. Barbados, St Kitts and Nevis accounted for almost 700,000 enslaved Africans (Table 1.3), representing just approximately 6.5% of the total number of disembarkations, based on 10,703,000 enslaved Africans entering the Americas between 1501-1867 (Eltis and Richardson, 2010).

Table 1.2. Showing the number of enslaved Africans taken to Nevis, St Kitts and Barbados and the eight main African regions sourcing the transatlantic slave trade, with the ten ports accounting for the majority of embarkation points for enslaved Africans. * Although no data are available for Luanda, this port supplied more enslaved Africans (EAs) to the Americas (including the Caribbean) than any other location in sub-Saharan Africa; ** Towards the end of the trade in enslaved Africans (from 1781 onwards), Quilimane grew rapidly as a port, until it became the largest port of embarkation outside the West Central Africa region. Data taken from Eltis and Richardson, 2010.

Port	Region	Country	Colonial	Barbados	St Kitts	Nevis
Anomabu	Gold Coast	Ghana	GB	31,000	3,300	8,700
Ouidah	Bight of Benin	Benin	France	59,000	800	5,100
Bonny	Bight of Biafra	Nigeria	GB	39,000	2,600	1,100
Old Calabar	Bight of Biafra	Nigeria	GB	28,000	1,700	3,100
Luanda*	West Cent. Af.	Angola	Portugal	_	_	_
Benguela	West Cent. Af.	Angola	Portugal	_	_	_
Cabinda	West Cent. Af.	Angola	France	6,000	5,000	1,500
Malembo	West Cent. Af.	Angola	France	10,000	4,300	900
Loango	West Cent. Af.	DRC	France	13,000	750	700
Quilimane**	SE Africa	Mozambiq.	Portugal	_	_	_

Table 1.3. Numbers of slaves registered on Barbados, St Kitts and Nevis and the percentages of the total number of recorded human disembarkations throughout the Americas between 1501-1867. Data taken from Eltis and Richardson, 2010.

	Number of slaves	Total (%) disembarkations
Barbados	503,041	4.7
St Kitts	139,139	1.3
Nevis	42,800	0.4

Approximately 80% of the enslaved Africans who disembarked in the Caribbean originated from just 20 African ports, with ten of these featuring heavily (Eltis and Richardson, 2010). Since becoming established, the Caribbean monkeys are the subject of both broad behavioural (Sade and Hildreth, 1965; Horrocks, 1982; Horrocks and Hunte, 1983), morphological (Ashton and Zuckerman, 1950; Ashton *et al*. 1979) and ecological (Coppinger and Maguire, 1980; Petto and Povinelli, 1985; Baula *et al.* 1987) studies and have been widely used in biomedical research (Van der Kuyl *et al*. 1996; Pandrea *et al*. 2006). Within the Caribbean, the introduced monkeys have a broadly omnivorous diet, largely consisting of agricultural fruits but also leaves, insects, reptiles, and even birds' eggs (Horrocks, 1982), living largely in habitats characterised by bush, scrub and xerophilic species (Petto and Povinelli, 1985). On St Kitts, the monkeys are thought to have been responsible for the extinction of the larger St Kitts bullfinch (*Loxigilla portoricensis grandis*) through the destruction of its nests and consumption of eggs (Sade and Hildreth, 1965). Whereas in Africa, *Chlorocebus* monkeys are predated on by chimpanzees, baboons, leopards, large raptors

and constricting snakes, the only natural predators in the Caribbean are red-tailed hawks, which are only able to take young and juvenile monkeys. They are also occasionally killed by dogs and are hunted by humans (Sade and Hildreth, 1965; Poirier, 1972; McGuire, 1974; Denham, 1981). On St Kitts, the minimum population is estimated to exceed 12,000 (Coppinger and Maguire, 1980), although the actual population was expected to be at least three times higher and on Barbados, the population size is thought to be approximately 25,000 animals (Poirier, 1972). On St Kitts and Barbados where the animals have been studied, the group size tends to be quite variable from 5 - 40 individuals (Coppinger and Maguire, 1980; Horrocks, 1982) and on all three islands, the monkeys are found in a broad range of habitats, from mountains and ravines, tropical deciduous forest, low acacia scrub and grasslands, and agricultural land (Poirier, 1972; Coppinger and Maguire, 1980).

1.5 Cranial anatomy

The skull is a vital structure, as it not only houses many of the sensory organs, such as the eyes and ears but it encases and protects the brain. The cranial vault comprises of the neurocranium, the area surrounding the brain, and the basicranium or skull base (Bourekas and Lanzieri, 1994) and encloses the brain. It is a relatively adynamic structure, in that it has no inherent potential to grow and changes only in response to brain growth. However, the cranial vault is still the source of a broad range of pathologies (Bourekas and Lanzieri ,1994; Aufderheide and Rodriguez-Martin, 2011). The skull base is made up of seven bones; paired frontal and temporal bones, and the unpaired ethmoid, sphenoid, and occipital bones (Fig. 1.4). It is divided into anterior, central, and posterior regions, which form the floor of the anterior, middle, and posterior cranial fossae (Baugnon and Hudgins, 2014). The basicranium is punctuated by numerous foramina, which serve various roles and functions. The largest of the skull base foramina is the foramen magnum, which is located within the posterior skull base, and transmits the medulla oblongata (cervi-comedullary junction), vertebral arteries and spinal portion of the cranial nerve CN XI (Baugnon and Hudgins, 2014). The posterior skull base is formed by the sphenoid, occipital, posterior-most part of the temporals and the parietals, and separates the posterior fossa structures, including the cerebellum and brainstem, from the extracranial soft tissues, such as the posterior nasopharynx, retro-pharyngeal space, carotid space, and perivertebral space (Baugnon and Hudgins, 2014).

In the craniofacial skeleton, growth involves a mosaic of specific sites that develop at different rates and mature at different times and overall, the skull responds to growth disruption in much more complex ways than that of the post-cranial skeleton. Different parts the craniofacial complex for example respond differently to the same hormonal or biomechanical stimulus (van den Berg *et al.* 2004a; Abbassy *et al.* 2008). The different regions of the cranium are characterised by different types of bone formation; the compact (cortical) bone surrounding the cranial vault for example is largely organised in parallel layers, known as lamellae and while lamellae are present in the cranial base, this area is instead typically comprised of more densely-packed trabecular 'spongy' bone, formed of very small layers organised into interconnecting struts (Lieberman, 2011). Once the skull has formed and undergone the appropriate morphogenetic transformations, it grows and develops to its appropriate size and shape and much of this development occurs along or near to sutures. Sutures are structures between the bones, formed by membranous ossification and are made of dense fibrous connective tissue and although points of fixation, the sutures are pliable and flexible, with growth occurring along their margins through osteoblastic activity (Bourekas and Lanzieri, 1994). Suture closure starts in the late juvenile stage and continues, until full obliteration, into adulthood. Sutural growth varies between different sutures and even within the same suture. Growth can be equal or unequal, with equal growth producing an even suture line and unequal growth leading to an irregular appearance (Barnes, 1994). Like all skeletal material, cranial bone is able to respond to

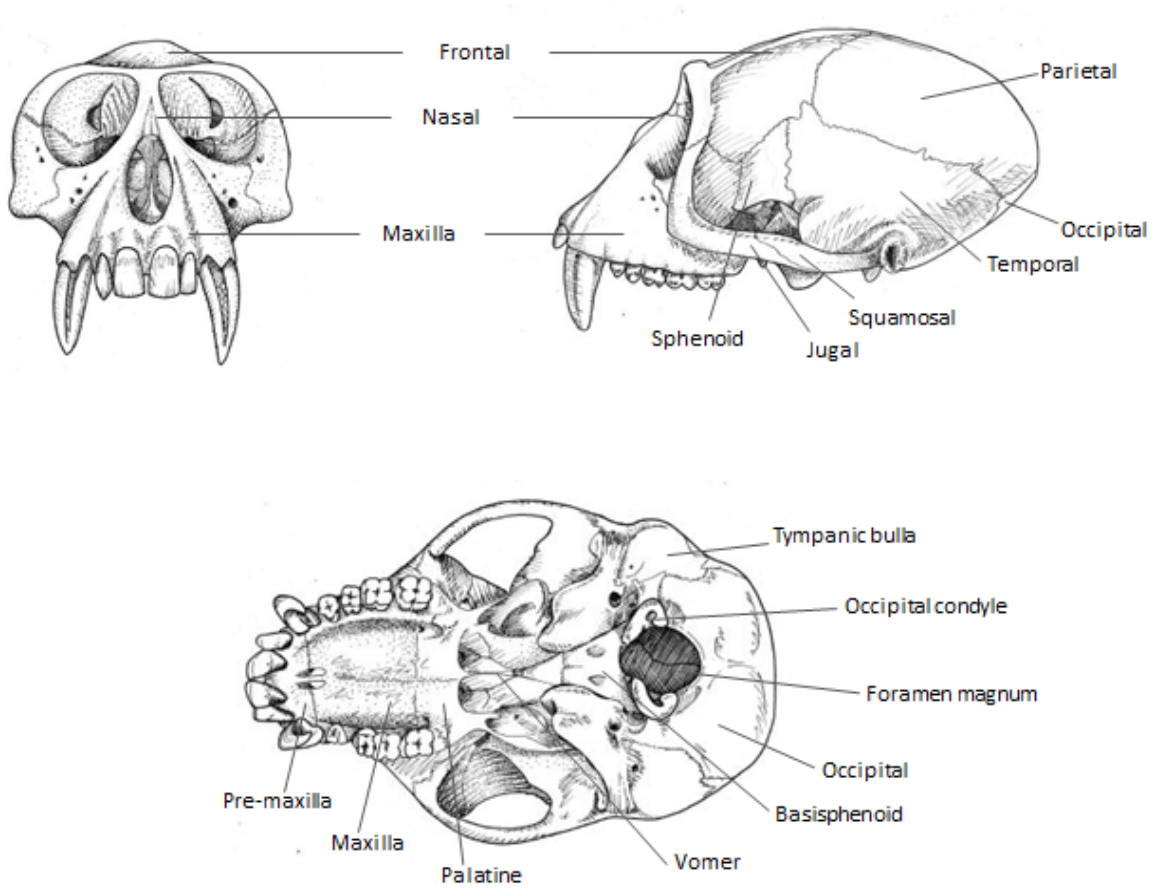

Figure 1.4. *Chlorocebus* cranium showing major cranial bones. Images in anterior (top left), lateral (top right) and ventral (bottom) aspects.

changes in mechanical loading (Lieberman, 2011). Bone is a relatively plastic tissue and whilst it does have a strong genetic component to its growth and development, a series of complex yet constrained interactions between bone

cells and the surrounding mechanical environment can influence bone morphology, particularly whilst the bone is still growing. The skeleton is continuously remodelled throughout an animal's life according to a complicated cascade of hypophyseal hormone feed-back mechanisms, vitamin activations and the continuous physical osteocytic mechano-transduction from daily loadings (Sonne *et al.* 2009) and whilst all bones are able to remodel under load force, sutures are not able to do the same (Rice and Sharpe, 2008).

1.6 Ancient DNA

The use of ancient DNA (aDNA) over the last two decades has become an invaluable analytical tool to investigate extinct species and populations (Callaway, 2011; Brace *et al*. 2014; Brace *et al*. 2015) and temporal genetic changes (Gilbert *et al*. 2005; Helgen *et al*. 2008; Carden *et al*. 2012; Langille *et al*. 2014). The techniques were originally designed to successfully extract the residual amounts of DNA remaining in samples that are hundreds or thousands of years old (Rohland and Hofreiter, 2007) and it was first used to extract DNA from the extinct quagga (*Equus quagga*) (Higuchi *et al*. 1984). In addition to aDNA techniques since being used to investigate extinct and iconic species such as the dodo (*Raphus cucullatus*) (Shapiro *et al*. 2002) mammoth (Höss *et al*. 1992; Hagelberg *et al*. 1994), aDNA is also a key analytical tool when samples are not necessarily old but may be heavily degraded, as in museum specimens for example (Pääbo et al. 2004), where damp and humid conditions may lead to the decomposition and loss of nucleotide sequence information. The use of polymerase chain reaction (PCR) combined with the use of aDNA techniques allows the salvation of information from samples in which the disintegration of DNA is not yet complete. Typically, samples subjected to aDNA techniques are degraded to a small average size, often between 100 to 500 base pairs (bp), due to both enzymatic processes after death and nonenzymatic hydrolytic cleavage of phosphodiester bonds in the phosphate-sugar backbone (Lindahl, 1993). Compared with contemporary DNA preparations from fresh tissue, aged material subjected to aDNA techniques are usually much shorter in

length. However, more recently-aged samples that may not even exceed 100 years old can also be subjected to aDNA techniques (Helgen et al. 2008; Carden et al. 2012), in instances where sample preservation may be poor such as in historic museum collections where environmental conditions are not controlled and where these conditions are often characterised as being warm and damp. In instances where fresh and 'old' material are directly compared, then because of variation in sample preservation and quality, all samples (both historic and modern) can be subjected to aDNA techniques in order to standardise the methods. In addition, a dedicated aDNA laboratory should be used in order to minimise the risks of sample contamination (Brace *et al*. 2012).

Analytical techniques using aDNA methods are subject to several potential areas of either ambiguity or inaccuracies. Because aDNA studies focus on samples where the DNA is expected to be heavily degraded (where only small fragments of DNA are available) and because the use of PCR allows for the amplification of a huge number of copies of the DNA, the risk of contamination is high. To counter this, standardised extraction and amplification methods (Yang *et al*. 1998; Rohland and Hofreiter, 2007; Jones *et al*. 2008) have been introduced and are widely followed, with guidelines designed to help ensure the high quality of aDNA data and conclusions (Cooper and Poinar, 2000) (Table 1.4).

Table 1.4. The nine criteria for authenticity, *from Cooper and Poinar, 2000*; designed to help ensure reliability and high standards in studies where aDNA is used.

(i) Isolation of work areas: to separate samples and extracted DNA from PCR amplified products.
(ii) Negative control extractions and amplifications: to screen for contaminants entering the process at any stage.
(iii) Appropriate molecular behaviour: owing to DNA degradation, the successful amplification of large DNA fragments in ancient DNA studies should be treated with caution.
(iv) Reproducibility: multiple PCR and extractions should yield consistent results.
(v) Cloning of products: to assess for damage, contamination and jumping PCR.
(vi) Independent replication: the generation of consistent results by independent research groups.
(vii) Biochemical preservation: preservation of other biomolecules that correlate with DNA survival (e.g. collagen or amino-acid racemization) should indicate good sample preservation.
(viii) Quantification: by competitive PCR or Real-Time PCR to give an indication of the number of starting templates in the reaction.
(ix) Association remains: are associated remains equally well preserved, and do they show evidence of contamination?

1.7 Island biogeography

The theory of island biogeography was first outlined by MacArthur and Wilson in 1963 and since its inception has been one of the most influential areas within ecological biogeography (Whittaker *et al*. 2008). Islands afford special opportunities to study speciation, dynamism of morphological traits, and the mechanisms that influence and drive changes in body size (Nowak *et al*. 2008; Losos and Ricklefs, 2009). The island biogeography theory focuses on many such dynamic fundamental processes operating on populations, in an attempt to explain emergent patterns of system species richness, turnover and endemism.

Island mammals for example often display remarkable evolutionary changes in size and morphology (Foster, 1964; Van Valen, 1973; Lomolino, 1985). Both theory and empirical data support the hypothesis that island mammals evolve at faster rates than their mainland congeners. It is also often assumed that the island effect is stronger and that evolution is faster on the smallest islands (Millien, 2009). The study of peripheral populations can be used to explain ecogeographic patterns and help determine the consequences of isolation and habitat fragmentation, and from shortly after the theory was first introduced, it has been applied to a wide range of insular systems, from oceanic islands and areas of montane habitats, to microcosms and even small ponds (Whittaker *et al*. 2008; Losos and Ricklefs, 2009). In what later became known as the 'Island Rule' (Van Valen, 1973; Meiri *et al*. 2008; Millien, 2009), Foster (1964) was the first to record the tendency of small mammals, such as rodents, to be larger on

islands when compared to their closest relatives on the mainland. In comparison, large mammals, such as deer and elephants, are often much smaller on islands than on the mainland (Heaney, 2007; Ingicco *et al*. 2014). The island rule appears to be a pervasive pattern, exhibited by mammals from a broad range of orders, functional groups and time periods (Lomolino, 2005; Benton *et al*. 2010; Chiozzi *et al*. 2014; Ingicco *et al*. 2014). In many instances, isolation on islands may lead to speciation between islands and the mainland, between islands in an archipelago, or even speciation within an island (Johnson *et al*. 2000). There remains, however, much scatter about the general trend.

Once introduced to an insular environment, a population may suddenly become subject to a whole host of different selective pressures (Table 1.5). Such populations often (especially during periods of initial colonization) go through population bottlenecks, a drop in population size as a result of a reduced range and the fixation of alleles (Raia and Meiri, 2006; Heaney, 2007; Nowak *et al*. 2008).

Evolution in insular populations is often thought to be closely related to characteristics of the islands and their mammalian faunas, such as island area, isolation and the presence or absence of carnivores (Meiri *et al*. 2008), in addition to intraspecific competition and resource availability (Nowak *et al*. 2008). Often, such insular-based changes are believed to be related to an increased ability to control more resources and enhance metabolic efficiency (Meiri *et al*. 2008). The tendency of mammals to increase or decrease body size

with respect to geography or time depends on the abundance, availability, and size of resources, in what may be termed the 'resource rule' (McNab, 2010).

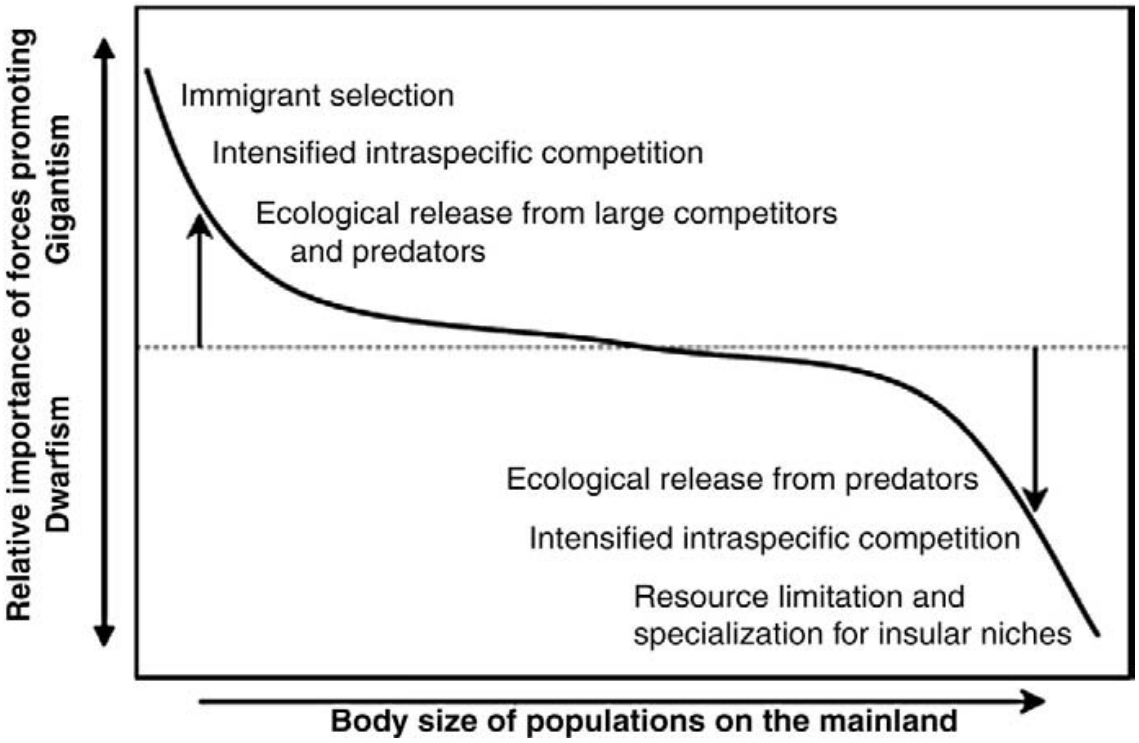

Table 1.5. In insular populations, the 'island rule' is an emergent pattern, which is often determined by a broad combination of selective pressures. These factors vary in their influence and importance on island populations in a predictable pattern along a gradient from small to relatively large species. From Benton *et al*. 2010.

Although the taxonomic status of many island primates is not always clear (Butynski *et al*. 2009), there is some evidence that primates are subject to the processes involved with insular speciation (Meiri *et al*. 2008; Shekelle, 2008), typically with dwarfism being observed (Bromham & Cardillo, 2007; Masters *et al*. 2014), including *Homo floresiensis* (Brown *et al*., 2004). Researchers have

undertaken relatively little work on primate isolates, but appreciating the effects of insularity and studying peripheral primate populations might be especially important as habitats become increasingly fragmented and, in essence, become islands (Nowak *et al*. 2008).

1.9 Species concepts

Although the concept of the term 'species' is of fundamental importance within biology, it is actually the point of long-running disagreement and continues to remain contentious today. The problem lies with the inability of biologists to agree on a single definition that can be applied to all naturally occurring organisms. As a consequence, numerous concepts have been posited in either an attempt to provide universal cover or to accommodate a particular taxonomic grouping (Table 1.6). In total, over 10 distinct definitions of species are commonly in use.

Table 1.6. The most commonly used species concepts; with concept definitions, strengths and weaknesses.

Species concept	Definition	Strengths	Weaknesses
Biological Species Concept (BSC) (Mayr, 1942; Hopf and Hopf 1985)	Groups of interbreeding (actually or potential) natural populations, which are reproductively isolated from other such groups.	This definition is a natural consequence of the effect of sexual reproduction on the dynamics of natural selection.	Only applies to sexually reproducing species. Not always possible to know whether two morphologically similar groups of organisms are potentially capable of interbreeding.
Phylogenetic Species Concept (PSC) (Wheeler and Platnick, 2000)	An evolutionarily divergent lineage, which has maintained its hereditary integrity with respect to other lineages through both time and space.	Effective means of determining levels of gene flow between populations. Enabled many new species to be identified that were not previously identified using other concepts.	If for example, only one polymorphic locus of a group of organisms is identified, organisms that should form distinct species could be clumped into one species.
Typological (Mayr, 1996; Cracraft, 2000)	Individuals sufficiently conform to certain fixed properties. Clusters of variations (phenotypes) differentiate species.	Classic method, still widely applied in research e.g. 'type method', where a single specimen ('types') is the basis for defining a species.	Different phenotypes do not always constitute different species e.g. sexual dimorphism, geographic variants.

Table 1.7. *continued*

Evolutionary (Wiley, 1978)	Single lineage of organisms within which genes can be shared, maintaining integrity with respect to other lineages.	Inherently morphological, but because morphologies have genetic bases, it indirectly includes a genetic component also.	Gaps in the fossil record impose arbitrary boundaries between species, especially those undergoing gradual size/shape evolution.
Ecological	Set of organisms adapted to a particular environmental niche.	Rather than focusing on discrete units, this concept focuses on selection, covering intermediary stages in speciation.	Supposes that naturally-occurring niches occupy discrete zones, with gaps between them.
Isolation	A set of actually or potentially interbreeding populations.	Useful when working with living examples of the higher taxa e.g. mammals, fish, birds.	Problematic for organisms that do not reproduce sexually.
Reproductive	Defined by organisms that are able to reproduce naturally to produce fertile offspring of both sexes.	May accurately represent what happens during the early stages of speciation.	Often difficult to know whether geographically isolated populations can potentially interbreed. Cannot be applied to fossils.
Cohesion (Templeton, 1989)	Population of individuals having the potential for phenotypic cohesion through intrinsic mechanisms.	Allows for post-mating isolation mechanisms. Allows for intermediate hybridization.	Dependent on genetic or demographic cohesion.
Genetic (Baker and Bradley, 2006)	A group of genetically compatible interbreeding natural populations that is genetically isolated from other such groups.	Recognition of species that are genetically isolated (but not reproductively isolated) results in an enhanced understanding of biodiversity and the nature of speciation.	Only works for sexually reproducing species. Does not cover fossil species.
Evolutionary Significant Unit (ESU) (Ryder, 1986)	A population of organisms considered distinct for purposes of conservation.	Can be used to conserve and maintain important populations with species.	Significant disagreement on what constitutes a single unit.

The most widely used (and generally agreed upon) concept used to define a species is the '*Biological Species Concept*' (BSC), which was introduced by Mayr in 1942. In this theory, species are defined as being groups that can interbreed with one another (or which can potentially interbreed), which form populations reproductively isolated from other such groups (Mayr, 1942; Hopf and Hopf 1985). Under the BSC, the emphasis is on those characteristics that tend to hold them together i.e. something that all members of a species have in common. Despite its common usage, there are various weaknesses in the concept. Under the BSC, species are those individuals that share a common method of fertilization, which, by definition, means that the concept only applies to sexually reproducing species. Additionally, due to either ethical or logistical considerations, researchers are often unable to determine whether two morphologically similar groups of organisms (which may or may not constitute species) are 'potentially' capable of interbreeding. Also, because there is considerable variation in the degree to which hybridization may succeed under natural conditions (even across genera), there are numerous ways where the central tenets of the concept are not adhered.

The other most commonly used idea is the 'Phylogenetic Species Concept' (PSC), which describes a species as an evolutionarily divergent lineage i.e. one that maintains its hereditary integrity with respect to other lineages through both time and space. At some point in the evolution of such a group, members may diverge from one another: when such a divergence becomes sufficiently clear, the two populations are regarded as separate species (Wheeler and Platnick,

2000). This category of species definition differs from many of the other species concepts in that parent of the phylogenetic species goes (taxonomically) extinct once a new species has evolved and instead, both parent and new populations are new species. One of the main problems with the PSC is that traits can only distinguish populations on a phylogeny once they have been isolated in terms of gene flow and have diverged genetically and/or morphologically. Additionally, the taxonomic level of subspecies is not recognized under this definition; anything under the species level is not taxonomically distinguishable.

1.10 The study of introduced island populations

Due to the multiple radiations, diversifications, extinctions, and recolonizations that have occurred over time and over space and because of increased opportunities to successfully combine morphology with mitochondrial phylogenetics in primate biogeography and speciation (Alfaro *et al*. 2015; Merces *et al*. 2015), many primate taxa have had their classification, phylogenetic relationships and biogeographic histories repeatedly discussed and revised, often with little consensus being reached (Morales-Jimenez *et al*. 2015).

The need for appropriate definitions of species limits are critical for both scientific study and conservation management (Parker *et al*. 2014), especially in areas where the possibility of speciation or a strong component of conservation management is in question. One area where this need is especially pertinent is in introduced or invasive insular populations and species. As with any local biotic community, introduced insular faunal assemblages typically arise via a successful invasion (or series of invasions) from a larger species pool, and are influenced via subsequent interactions with resident species such as predators, prey, and competitors (Donlan and Wilcox, 2008). This process of ecological invasive establishment is often associated with subsequent species decline and extinctions, and nowhere is this more apparent than in insular ecosystems (Donlan and Wilcox, 2008; Barun *et al*. 2013). Over the last 500 years, introduced species are thought to have been responsible for more documented vertebrate extinctions worldwide than any other agent (Donlan and Wilcox,

2008; Helgen *et al*. 2008), with such invasive species being able to fundamentally and unpredictably transform the ecological dynamics of whole ecosystems.

The reconstruction and veracity of introduction history for invasive species is crucial in understanding the evolutionary ecology of such species. Traditionally, the ability to reconstruct the introduction history for invasive species depended on often sparse or non-existent written records (Purcell *et al*. 2012). However, even when written historical records are available, the reliability of such introduction reports is critical in order to develop effective management recommendations for conservation actions. Identifying the sources, roots and order of introductions allows us to understand the processes involved in insular species evolution and for relevant measures of conservation or control to be designed and implemented (Rollins *et al*. 2009). Understanding the history of introductions and impacts of founder events on invasive species is crucial to understanding the evolutionary mechanisms driving successful invasions.

However, there has been an increased discussion surrounding the seemingly paradoxical nature of invasion founder events and how introduced populations are often highly successful despite their presumed limited genetic diversity (Purcell *et al*. 2012). Molecular studies can be used to support or counter the credibility of not only historical introduction records (Barun *et al*. 2013) but also studies based on morphological or behavioural studies alone (Alfaro *et al*. 2015; Solózarno-García *et al*. 2015). The presence of an insular population where the

individuals display intermediate characters between two closely related species can lead to problems in the identification of taxa and may compromise the species concept used, especially when genetic markers identify alternative species boundaries (Gillespie *et al*. 2013). This can lead to false conclusions about allopatric speciation, especially in island populations where differentiation at the specific level may be subjective.

Whilst studies rarely look at whether phylogenetic data supports or contradicts historical data, many recent studies have used inferences based in part on patterns of genetic variation to reconstruct the history of invasion of an introduced species (Barun *et al*. 2013), comparing introduced populations or species to the original populations or species from which they originated. Whilst helping to elucidate processes of speciation, the molecular narrative behind insular introductions is however beset with apparent paradoxes. Island colonisers may experience relaxed selective constraints, allowing the fixation of more slightly deleterious mutations, which would otherwise be actively selected against. Many habitat niches are often underexploited during the early stages of island colonisation, which leads to a reduction in resource competition. Additionally, colonisers often experience a relaxation in many selective pressures, resulting from there being fewer competitors or predators, which would otherwise be present (Woolfit and Bromham, 2005).

When compared to source populations, heterozygosity is often reduced in introduced populations (Purcell *et al*. 2012) but a small founder population size

does not necessarily mean that invasive populations are characterized by a reduced genetic diversity (Barun *et al*. 2013). However, when a subset of a species is reproductively isolated, its divergence is often accelerated because of the increased genetic drift that can lead to founder effect phenomena when genetic diversity is low in small populations (Templeton, 1980; Okada *et al*. 2014). Introduced insular species in particular tend to have significantly higher ratios of non-synonymous to synonymous substitution rates than their mainland relatives. This is best explained by an increase in nearly neutral mutations drifting to fixation in small island populations (Woolfit and Bromham, 2005). The early stage population dynamics of colonisers play a major role in determining how much genetic diversity is retained within and among populations (Barun *et al*. 2013).

A population that increases in size rapidly after a founder event for example will lose relatively little variation, whereas substantial variation can be lost when the founder population remains small for several generations. Considering that mutation often has a minimal or negligible influence given the age of most biological invasions, the genetic variation observed in introduced populations often depends strongly on the past history of the invasive/introduced species within its native range (Barun *et al*. 2013). This variation is dependent on a range of biotic factors such as propagule pressure, drift, and sometimes natural selection. Therefore, to more fully understand the processes involved with colonisation, it is necessary to understand the historical patterns of genetic diversity within the native range (Taylor and Keller, 2007).

Chapter 2

A Phylogenetic Monkey Puzzle Tree: reassessing the mitochondrial diversity of African *Chlorocebus* and investigating how Caribbean *Chlorocebus* monkeys sit within this phylogeny.

2.1 Introduction

Due to the multiple radiations, diversifications, extinctions, and recolonizations that have occurred over time and because of increased opportunities to successfully combine morphology with mitochondrial phylogenetics in primate biogeography (Alfaro *et al*. 2015; Merces *et al*. 2015), many primate taxa have had their classification, phylogenetic relationships and biogeographic histories repeatedly discussed and revised, often with little consensus being reached (Morales-Jimenez *et al*. 2015). The need for appropriate definitions of species limits are critical for both scientific study and conservation management (Parker *et al*. 2014), especially where the possibility of speciation or a strong component of conservation management is in question. One area where this need is especially pertinent is in introduced or invasive insular populations and species. As with any local biotic community, introduced insular faunal assemblages typically arise via a successful invasion (or series of invasions) from a larger species pool (Mayr, 1942; Coyne, 1994) and are influenced by subsequent interactions with resident species such as predators, prey, and competitors (Thornton, 2007; Donlan and Wilcox, 2008). This process of ecological invasive establishment is often associated with subsequent species decline and extinctions, and nowhere is this more apparent than in insular ecosystems (Donlan and Wilcox, 2008; Barun *et al*. 2013). Such reproductive isolation on island ecosystems allows for the rapid acceleration in a population's divergence due to an increased genetic drift. This increase may then lead to founder effect events in populations where genetic variation is limited (Okada *et al*. 2014). Over the last 500 years, introduced species are thought to have been

responsible for more documented vertebrate extinctions worldwide than any other agent (Donlan and Wilcox, 2008; Helgen *et al*. 2008), with such invasive species being able to fundamentally and unpredictably transform the ecological dynamics of whole ecosystems (Thornton, 2007; Turvey, 2009).

The reconstruction of introduction history for invasive species is crucial in understanding the evolutionary ecology of such species. Traditionally, the ability to reconstruct the introduction history for invasive species depended on often sparse or non-existent written records (Purcell *et al*. 2012). However, even when written historical records are available, the reliability of such introduction reports is often critical in order to develop effective management recommendations for conservation actions. Identifying the sources, roots and order of introductions allows us to understand the processes involved in insular species evolution and for relevant measures of conservation or control to be designed and implemented (Rollins *et al*. 2009). Understanding the history of introductions and impacts of founder events on invasive species is crucial to understanding the evolutionary mechanisms driving successful invasions. However, there has been an increased discussion surrounding the seemingly paradoxical nature of invasion founder events and how introduced populations are often highly successful despite their presumed limited genetic diversity (Purcell *et al*. 2012). Molecular studies can be used to support or counter the credibility of not only historical introduction records (Barun *et al*. 2013) but also studies based on morphological or behavioural studies alone (Alfaro *et al*. 2015; Solózarno-García *et al*. 2015). The presence of an insular population where the individuals display intermediate characters between two closely related species

can lead to problems in the identification of taxa and may compromise the species concept used, especially when genetic markers identify alternative species boundaries (Gillespie *et al*. 2013). This can lead to false conclusions about allopatric speciation, especially in island populations where differentiation at the specific level may be subjective.

Whilst studies rarely look at whether phylogenetic data supports or contradicts historical data, many recent studies have used inferences based, in part, on patterns of genetic variation to reconstruct the history of invasion of an introduced species (Hufbauer *et al*. 2004; Kawamura *et al*. 2006; Thulin *et al*. 2006; Estoup *et al*. 2010; Barun *et al*. 2013), comparing introduced populations or species to the original populations or species from which they originated. Whilst helping to elucidate processes of speciation, the molecular history narrative behind insular introductions is however beset with apparent paradoxes. Island colonisers may experience relaxed selective constraints, causing deleterious mutations, which would otherwise be actively selected against. Many habitat niches are often underexploited during the early stages of island colonisation, which leads to a reduction in resource competition. Additionally, colonisers often experience a relaxation in many selective pressures, resulting from there being fewer competitors or predators, which would otherwise be present (Woolfit and Bromham, 2005).

Green monkeys (*Chlorocebus* spp.) were transported from Africa to the Caribbean between the 17-19th centuries, with multiple introductions occurring

between 1627-1807 (Denham, 1987). Introduced to the islands of St Kitts, Nevis and Barbados, it is unclear as to whether the monkeys were taken solely to be sold as pets or for the purposes of eating. It seems likely however, that the pet trade would have largely fuelled their transportation. Similarly, it is unclear exactly when feral breeding populations originated in the Caribbean but there was already a bounty on their heads by 1682, as they represented a significant agricultural threat through crop-raiding (McGuire, 1974; Denham, 1987). Within the Caribbean, *Chlorocebus* monkeys are collectively known as 'African green monkeys' (AGMs) and from historical accounts and records such as early colonial reports and slave ship log books, they have long been considered to be coastal West African in their origin, being transported in their hundreds from multiple ports from Senegal, Gambia, Gabon, Nigeria and as far south as Angola and South Africa (McGuire, 1974; Denham, 1987). Recent molecular results however show that the St Kitts population are phylogenetically clustered, appearing to represent a population stemming from a single source population (Brown *et al*. 2013). Using cytochrome *b* (Cyt *b*) sequencing, the St Kitts monkeys have been confirmed as being African green monkeys (*Chlorocebus sabaeus*), most likely originating from Senegal (Van der Kuyl *et al*. 1996; Pandrea *et al*. 2006). From here, is generally thought that all Caribbean monkeys have a similar phylogeny. Because a more complete study has not been carried out on all the Caribbean populations of *Chlorocebus* and because the taxonomic status of the various African-based *Chlorocebus* groups is still the subject of debate, there remains many questions regarding both Caribbean and African *Chlorocebus*.

Across much of Africa, Plio-Pleistocene climate change (starting approximately 5.3 Ma) caused dramatic shifts in faunal assemblages (Bobe and Behrensmeyer, 2004; deMenocal, 2004), and a combination of lower temperatures coupled with increased aridity in East Africa reduced and fragmented tropical forests, leaving them as isolated patches along major rivers and areas of high elevation (Bobe and Behrensmeyer, 2004). This creation of a patchwork of forested habitats has contributed in part to the rapid evolution and diversity of the African guenons (tribe: Cercopithecini) and continues to do so today (Kamilar *et al*. 2009). Primate populations which have undergone recent but significant habitat loss and fragmentation, show low levels of genetic diversity, due to elevated genetic drift (Mbora and McPeek, 2010) and in those species characterised by female philopatry, a mitochondrial DNA (mtDNA) pattern which is relatively homogenised within populations, as is found in forest patches for example, but heterogenous between populations (Shimada, 2000; Mbora and McPeek, 2010) is typically seen. These discrete habitats act as a mechanism of geographic isolation and it is probable that recent phylogeographic history has shaped the pattern of genetic differentiation (Ming *et al*. 2007) in many of the African guenons. Although there is often a level of discordance between mitochondrial phylogeny and morphology, many other African non-human primates such as *Papio*, can be broken down into several well-supported major haplogroups, reflecting distinct geographic populations (Zinner *et al*. 2009). The guenons are a species-rich group of African monkeys and provide an ideal model for investigating areas such as speciation and the processes involved in colonisation within evolutionary biology (Kamilar *et al*. 2009). The group includes subsets of taxa where some species and populations are characterized by having overlapping ranges, whilst others are

geographically separated, making detailed comparisons possible. Additionally, range maps are known for the vast majority of cercopithecines and because some guenon species are widely used in biomedical research, their molecular profiles are well studied. There is however very often a sizeable level of discord between morphological and molecular traits in terms of establishing taxonomic status when looking at either mtDNA and/or nuclear DNA (nDNA) in the wider group of cercopithecines (Detwiler *et al*. 2005; Zinner *et al*. 2011) and the *Chlorocebus* clade is no exception. Recent molecular work focusing on the mitochondrial diversity of African green monkeys (Haus *et al*. 2013) showed that mtDNA diversity does not conform to the existing taxonomic classification of the group and that in several instances there were clear examples of disparity between phenotypes and mtDNA status, possibly relating to probable contact zone hybridisations. The *Chlorocebus* genus is currently interpreted as a sister taxon to two other ground-dwelling, semi-arboreal cercopithecine groups; the patas monkey (*Erythrocebus*) and the L'Hoest's monkey (*Allochrocebus*). There is also currently still much debate with regards the classification of the *Chlorocebus*: namely, whether they are one polytypic species (*Chlorocebus aethiops*) and are subdivided into several subspecies (Kingdon, 1997; Elton *et al*. 2010), or whether they form six distinct species (Groves, 2001): green monkey (*Chlorocebus sabaeus*), tantalus monkey, (*C. tantalus*), malbrouck monkey, (*C. cynosuros*), vervet monkey, (*C. pygerythrus*), grivet monkey (*C. aethiops*) and Bale monkey (*C. djamdjamensis*), with both *C. tantalus* and *C. pygerythrus* being polytypic (Fig 2.1).

Caribbean-based *Chlorocebus* monkeys represent an important area for study. They are used extensively in biomedical (AIDS) research, to investigate both viral replication and immune responses in natural hosts infected with SIV (Pandrea *et al*. 2006). Although each African *Chlorocebus* species has its own distinct SIV subtype in the wild, Caribbean laboratory animals are all infected with the SIVagm.sab subtype (originating in *C. sabaeus* only). All subsequent health monitoring and treatment is based on the idea that all Caribbean *Chlorocebus* monkeys are derived from African green monkeys (*C. sabaeus*) from a restricted area in the Senegambia (Senegal-Gambia) region (Baulu *et al*. 2002; Pandrea *et al*. 2006). If however, Caribbean *Chlorocebus* monkeys do not all originate from *C. sabaeus*, then this may have a series of negative consequences not only for the reliability of previously published biomedical data but potentially also for the efficacy of any treatments derived from this Caribbean population. A previous study (Alfaro *et al*. 2015) sampled 17 individuals from two Caribbean populations (Barbados and St Kitts) and compared them with 14 individuals from three *Chlorocebus* species in Africa (from the Central African Republic, Tanzania and Senegal).

Additionally, they represent a medium-sized invasive species that was introduced into the West Indies, where approximately 90% of terrestrial mammal species have been extirpated in recent times (Alfaro *et al*. 2015). Understanding how these monkeys became established in the Caribbean may further not only our understanding of the processes involved with insular colonisation but may also reveal how a multidisciplinary approach may be used

to understand these events.

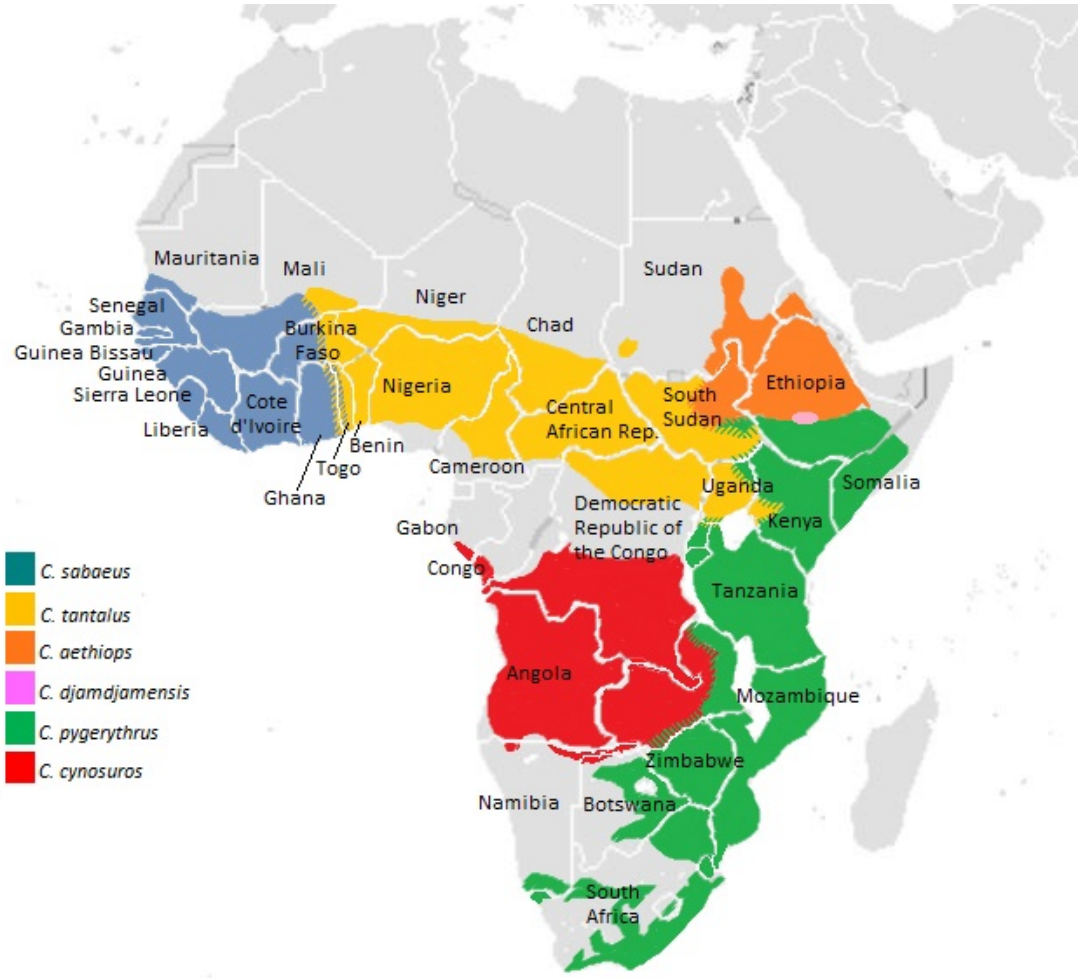

Figure 2.1. Map of Africa, showing the distribution of the six *Chlorocebus* species in their sub-Saharan ranges. Despite being excluded from much of the forested Congo basin and southern African forests, African green monkeys are found across numerous habitats. Four species (*C. sabaeus, C. tantalus, C. cynosuros* and *C. pygerythrus*) have ranges along the western coast, coinciding with many historical ports used by slavers.

It has been suggested that genetic lineages represented by founder monkeys imported from other parts of Africa may have been lost, partly due to inbreeding (Van der Kuyl *et al*. 1996). Given that there is a largely-unresolved phylogeny of the African *Chlorocebus* clade, that few phylogenetic studies have been performed on the *Chlorocebus* populations within the Caribbean, and that

Caribbean *Chlorocebus* monkeys are used in biomedical research, this chapter investigates whether (1) the phylogeny supports the current species designations in the African *Chlorocebus* species; (2) whether the Caribbean populations of *Chlorocebus* represent a single or multiple colonisation event, and (3) if the Caribbean *Chlorocebus* monkeys originate from a single or multiple African source population.

2.2 Methods

2.2.1 Sample collection

In order to investigate the phylogeographic status of the *Chlorocebus* clade, a total of 73 samples were successfully collected (*Appendix 1*): 24 from Genbank, 39 from museum collections and 10 from wild populations in the Caribbean. Samples were collected from each of the three Caribbean island populations (St Kitts, Nevis and Barbados) and the three species of *Chlorocebus* found along the western coast of Africa (*C. sabaeus, C. tantalus* and *C. pygerythrus*). In addition to samples taken directly for the study, data were collected from Genbank (n=24) to complement collected material (and to include the three other African species). Samples were taken from museum specimens only where there was a reliable provenance (and taxonomic status), along with the country of origin. Whilst some specimen labels gave very specific locality data (such as named forest blocks), the vast majority only gave the country of origin, preventing a more comprehensive understanding of sample origins.
Considering that the taxonomy of *Chlorocebus* has undergone several major revisions, only clearly labelled specimens were used. Specimens were recorded following the standardised *Chlorocebus* taxonomy (Kingdon, 1997; Groves 2001). Museum samples were used throughout this study. The use of such material permitted a much broader set of samples to be used from a larger geographical range (Farrell *et al*. 2015), in a more affordable and expedient manner.
When extracting DNA from museum specimens, ancient DNA (aDNA)

extraction techniques were employed each time (Brace *et al*. 2014; Brace *et al*. 2015). In order to reduce the possibility of varying methods affecting either the efficacy of extraction or the results, the same method was used for all modern, field derived, samples. Wherever possible, drilling was carried out on one of either the molars or premolars. This was done partly because these teeth are often loose and invasive sampling can be carried out with minimal impact to either the overall structural integrity of the skull or upon potential future uses of the skull such as the application of morphological landmarks. Also, sampling the teeth allows a high yield of mtDNA to be extracted, when compared to other sites across the skeleton (Adler *et al*., 2011; Rohland and Hofreiter, 2007a). Specifically, the root of the tooth is especially good for DNA extraction, with the yield being up to five times greater than elsewhere (Adler *et al*. 2011). Prior to drilling, the surface of the tooth was cleaned with a tissue, as dirt is known to introduce a number of inhibitory substances to the extraction process (Rohland and Hofreiter, 2007a). For each sample, a new drill-bit was used. Before drilling, both the drill-bit and all surrounding surfaces were washed using a dilute household bleach NaOCl solution. Each drill-bit was also immersed in sterile water and was dipped in ethanol ($CH_3\ CH_2\ OH$) before being passed through a flame to burn off any residual alcohol. Samples were collected on UV-treated aluminium foil (Adler *et al*. 2011) and transferred to sterile, single-use eppendorfs. Latex gloves were worn throughout the whole process, with a new pair being used for each extraction. For drilling, a 1.5mm drill-bit was used for sampling each time, along with a variable-speed Li-ion Sparky Professional drill. Running a drill at ‘normal’ speed (c. 1000 RPM) has been shown to reduce the mtDNA yield up to 30 times (Adler *et al*. 2011; Rohland and Hofreiter, 2007b),

due to effects of heat damage. Instead, very low speed (c. 100-200 RPM) was used for sampling, as this speed has been shown to have no impact on the quality or quantity of the yield. For each sample, 20mg of drilled bone/tooth was collected. Samples collected from the Caribbean (Nevis and Barbados only) were taken from freshly killed animals, which had been culled as part of an ongoing, government-led programme. All research on this project complied with ethical protocols and procedures set out by University College London, the Zoological Society of London, and the Barbados Primate Research Centre and Wildlife Reserve, and the Nevis Ministry of Agriculture, Marine Resources and Cooperatives.

2.2.2 Extraction, Amplification, and Sequencing of DNA

Because of variation in sample preservation and quality and in order to avoid contamination with modern DNA, all extractions (museum and field) were conducted in a laboratory dedicated to ancient DNA analysis at Royal Holloway, University of London. To assess any possible cross-contamination of samples, two blank extractions (without samples) were processed per batch (6-12) of samples. Extraction of DNA material from bone/tooth samples was performed with the Qiagen PCR Purification Kit (Qiagen, Germany), following standardised protocols (Brace *et al*. 2012), with the only differences being the replication of specific steps to accommodate aDNA methods. Due to the degraded nature of the museum samples (Brace *et al*. 2012), a reduced section of the mitochondrial gene was used for amplification and two hundred and fifty nine (259) bp from the Cyt *b* region were PCR amplified with primer pairs designed

for this study (Table 2.1). Rather than nDNA, this study used mtDNA as it has been identified as an ideal marker to assess species and subspecies relationships, as it displays extensive intraspecific polymorphism (Šanda *et al*. 2014), evolves faster than nDNA and is present at a higher copy number (Brace *et al*. 2012). Additionally, in mammalian species showing female philopatry (as is seen in *Chlorocebus*) mtDNA is known to conserve geographical pattern better than nuclear DNA (Avise, 2009; Haus *et al*. 2013). Within the Caribbean population, successful extraction, amplification and sequencing was only possible for St Kitts and Barbados. Samples from Nevis did not yield material for successful extractions.

Table 2.1. Primers designed and used and their bases.

Primer	Bases
PAf (*Primer A forward*)	TCACCAGACACCTCTTCTGC
Par (*Primer A reverse*)	CTCAGAATGATATTTGGCCTCA
PBf (*Primer B forward*)	GCCTCCATATTTTTCATCTGCC
PBr (*Primer B reverse*)	ATTCATTGGACGAGGTCGGT

Amplification followed a standard procedure (Yang *et al*. 1998) and was performed in a total volume of 25.0µl; containing 15.1µl purified water, 2.5µl 10x buffer, 2.5µl 1% BSA, 0.5µl MgCl2, 0.2µl dNTPs, 1.0µl of each primer, 0.2µl Taq (HotStar) and 2.0µl sample. Amplifications were performed with pre-denaturing at 95 °C for 5 min, fifty cycles for denaturing at 94 °C for 60s, annealing at 56 °C for 60s, extension at 72 °C for 60s, and a final 10min of extension at 72 °C. Reactions were conducted with one or two blanks in

addition to the extraction blanks. All negative controls were clean. Sequencing reactions were performed by Macrogen (South Korea) using a high-throughput genetic analysis sequencer (ABI3730XL). Sequencing chromatograms were assembled and analysed using SEQUENCHER v4.7 analysis software (Gene Codes Corporation, Ann Arbor, Michigan, USA). Throughout procedures, protocols to prevent contamination and ensure accurately coded (undamaged) mtDNA were followed: geographic isolation of work areas, multiple negative controls, observation of reduced fragment length amplification and appropriate molecular behaviour, and repeated amplification and sequencing of fragments (Brace *et al*. 2012). Repeat PCRs were performed using a final concentration of 10x PCR buffer, 10 µm of each primer, 25 µm dNTPs, 2 mM $MgSO_4$, 1 mg/mL BSA, 1Unit Platinum® *Taq* DNA Polymerase High Fidelity, purified water, and 2 µL of DNA extract in a 25-µL mix. PCR conditions were 5 min at 95 °C, followed by 40 cycles of 1 min at 92 °C, 1 min at 48 °C, 1 min at 68 °C, and with a final extension of 5 min at 68 °C.

2.2.3 Statistical analyses

Chlorocebus sequences were assembled and aligned using the programme Se-Al 2.0 (Rambaut, 1996) and were corrected by eye and using Sequencher 4.7. For each individual sample, forward and reverse sequencing was performed. Phylogenetic relationships were estimated using maximum likelihood (ML) and Bayesian methods. Of the 59 *Chlorocebus* specimens sampled, 49 successfully yielded DNA (83.05% success). Of these, 39 samples were from museums and 10 were from freshly collected Caribbean samples. In addition to the 49 successful samples, an additional 24 sequences were taken from Genbank. In

order to construct a meaningful phylogenetic tree, the generated sequence data was run through PartitionFinder (v1.1.1) (Lanfear *et al*. 2012), in order to see which tree-building model gave the best fit, by looking at each codon position. The data were run through a Bayesian tree-building programme (Mr Bayes v3.2.0) (Huelsenback and Ronquist, 2001), taking into consideration that Cyt *b* often shows differing levels of variation depending on codon position, due to the redundancy inherent in the triplet code. The software assesses the position of the base pair within the codon, in order to construct the most parsimonious tree. The data was assessed as a whole and the appropriate nucleotide substitution model needed to build the phylogenetic tree was assessed with JMODELTEST 3.7 (Posada and Crandll, 1998). The Tamura-Nei (TrN) model (with gamma distribution - shape parameter 0.0078) was selected as being the most appropriate model. This model assumes variable base frequencies, equal transversion rates and variable transition rates (Tamura *et al*. 2011). Nodal support was determined using ML bootstrap analysis with 1 x 10^3 replicates in MEGA.5 (Madisch *et al*. 2007; Tamura *et al*. 2011; Rogers *et al*. 2012; Brace *et al*. 2014) and tree trees were created in PAUP* (Swofford, 2000) using a full heuristic search with branch swapping by tree-bisecting-reconnection (TBR). *Cercopithecus mona* was used as an outgroup for both sets of analyses. Nodal support was determined using ML bootstrap analysis with 1000 replicates in PAUP*.

2.3 Results

In constructing the phylogenetic tree for *Chlorocebus*, data from 73 sequences were available; 39 from museums, 10 samples from Caribbean field collection and 24 samples from Genbank, thus covering the six African *Chlorocebus* species and the Caribbean populations on St Kitts and Barbados (Table 2.2).

Table 2.2. Number of *Chlorocebus* sequences obtained for analysis, including Caribbean samples from Barbados and St Kitts.

Species / Population	Source	No. sequences
C. sabaeus	B.Faso, S.Leone, Ghana, Senegal	11
C. tantalus	Cameroon, CAR, Nigeria	11
C. aethiops	Ethiopia	4
C.djamdjamensis	Ethiopia	3
C. pygerythrus	Ethiopia, Kenya, Tanzania, S.Africa	12
C. cynosuros	Zambia, Angola, DRC	3
Caribbean	Barbados	10
Caribbean	St Kitts	19

After constructing several trees, the presence of five haplotypes (Table 2.3) gave these initial trees a crowded appearance and clear division amongst many of the clades was not evident (Fig 2.2). As a result, these haplotypes were condensed (using FaBox 1.41) into single branches (Fig 2.3). Sequences from Genbank often represented the whole mitochondrial genome and were thus much longer (16,438 bp) than those samples obtained for this study from museums or the Caribbean, where sequences were often from degraded samples. These longer samples were cut down, so that all samples were of a

comparable length (260 bp). The mona monkey (*Cercopithecus mona*) was used as an outgroup for the phylogeny.

Table 2.3. Haplotypes and their component samples appearing in the phylogenetic tree in this study. Also shown are the country of origin and the source for each sample.

Haplotype name in tree	Seq.	Country of origin	Sample source
B033_Haplo_2Nigeria	B033	Nigeria	Royal Coll. Surgeons, London
	B034	Nigeria	Royal Coll. Surgeons, London
B009_Haplo_24	B008	St. Kitts	Royal Coll. Surgeons, London
	B009	St. Kitts	Royal Coll. Surgeons, London
	B010	St. Kitts	Royal Coll. Surgeons, London
	B011	St. Kitts	Royal Coll. Surgeons, London
	B014	St. Kitts	Royal Coll. Surgeons, London
	B015	St. Kitts	Royal Coll. Surgeons, London
	B016	St. Kitts	Royal Coll. Surgeons, London
	B021	St. Kitts	Royal Coll. Surgeons, London
	B022	St. Kitts	Royal Coll. Surgeons, London
	B023	St. Kitts	Royal Coll. Surgeons, London
	B024	St. Kitts	Royal Coll. Surgeons, London
	B025	St. Kitts	Royal Coll. Surgeons, London
	B039	St. Kitts	Royal Coll. Surgeons, London
	B040	St. Kitts	Royal Coll. Surgeons, London
	B062	Barbados	Field - Barbados
	B064	Barbados	Field - Barbados
	B065	Barbados	Field - Barbados
	B066	Barbados	Field - Barbados
	B067	Barbados	Field - Barbados
	B068	Barbados	Field – Barbados
	BG005	Nigeria	Royal Coll. Surgeons, London
	Ang_4*	Nigeria	Royal Coll. Surgeons, London
	BG020	S. Leone	Royal Coll. Surgeons, London
	BG038	S. Leone	Royal Coll. Surgeons, London
	BG026	Zimbabwe	Royal Coll. Surgeons, London
	BG029	Zimbabwe	Royal Coll. Surgeons, London
	BG041	Senegal	Royal Coll. Surgeons, London
B013_Haplo_KKKK	B013	St. Kitts	Royal Coll. Surgeons, London
	B018	St. Kitts	Royal Coll. Surgeons, London
	B028	St. Kitts	Royal Coll. Surgeons, London
	B030	St. Kitts	Royal Coll. Surgeons, London
B006_Haplo_N_B	B006	Nigeria	Royal Coll. Surgeons, London
	B059	Barbados	Royal Coll. Surgeons, London
B002_Haplo_K_ZZZZ	B002	St. Kitts	Royal Coll. Surgeons, London
	B012	Zimbabwe	Royal Coll. Surgeons, London
	B017	Zimbabwe	Royal Coll. Surgeons, London

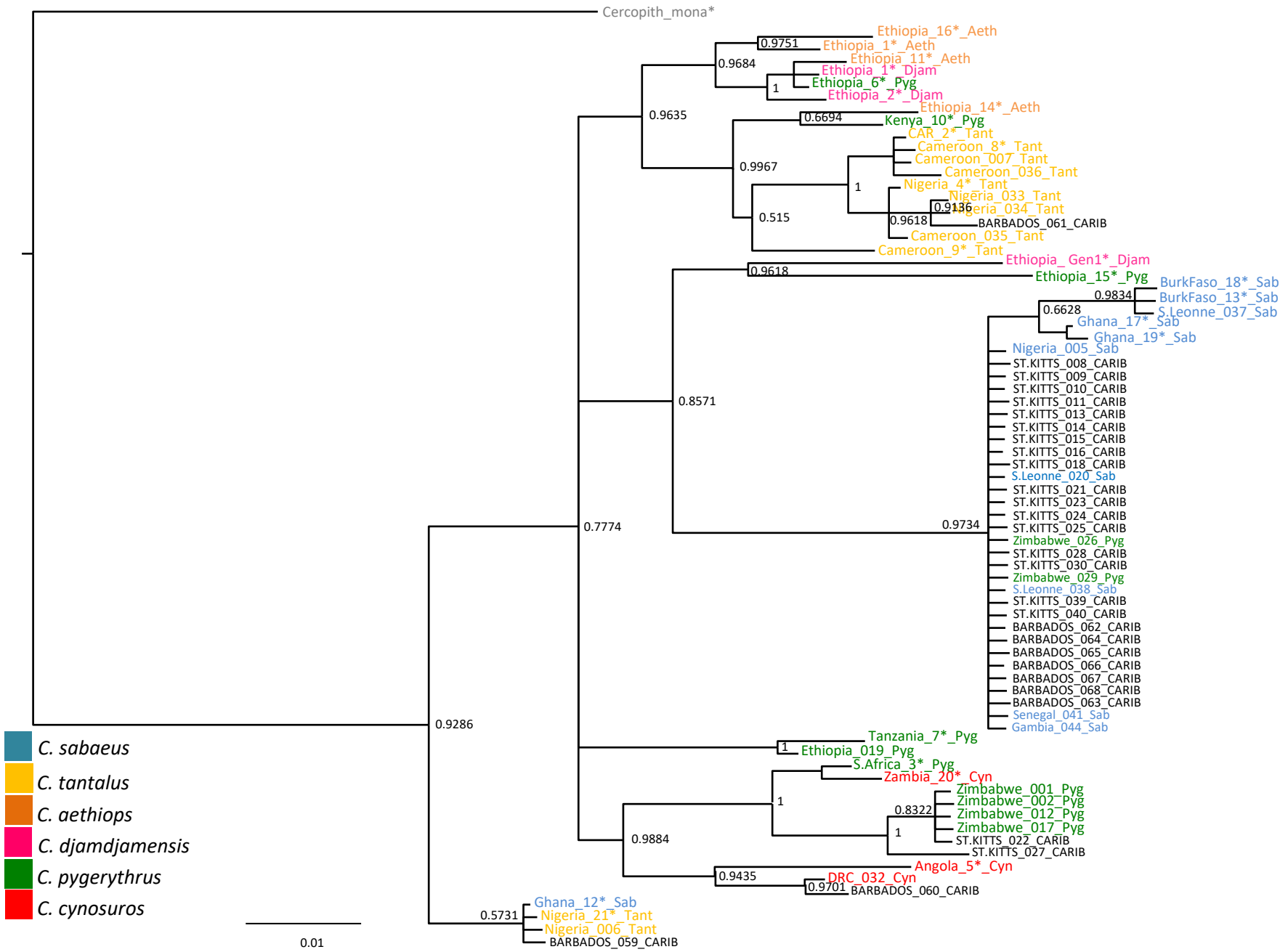

Figure 2.2. *Chlorocebus* phylogeny inferred from mtDNA (cyt b) sequence data showing Bayesian probabilities of nodes. Caribbean sequences are in black and * denotes a sequence from Genbank. C 1-3 shows major phylogenetic clades. Scale bar represents number of substitutions per site.

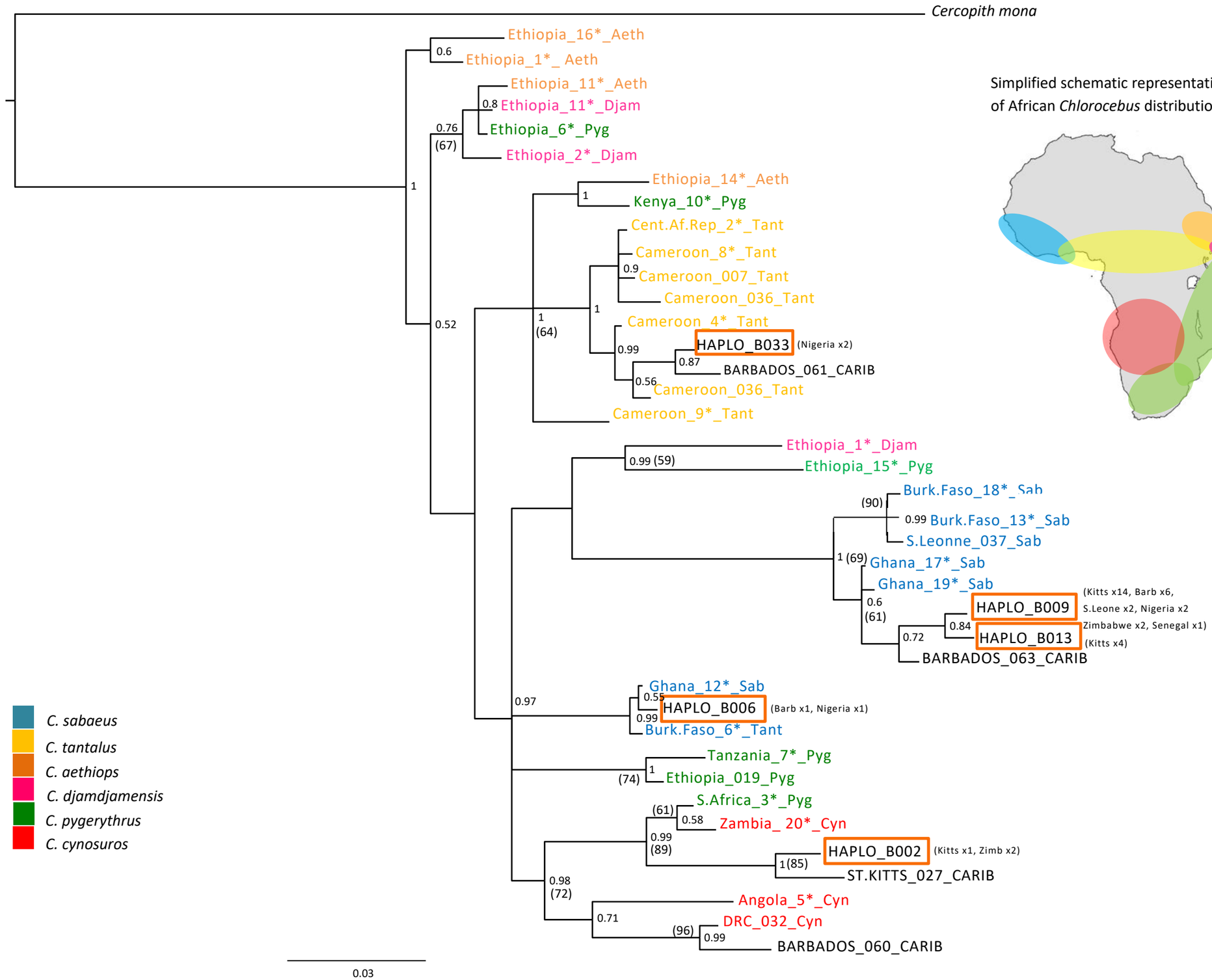

Figure 2.3. *Chlorocebus* phylogeny inferred from mtDNA (cyt b) sequence data showing Bayesian probabilities of nodes where only Bootstrap values of 50% and over are shown, with haplotypes (boxed in red) collapsed. Caribbean sequences are in black and * denotes a sequence from Genbank. Scale bar represents number of substitutions per site.

Maximum likelihood (Fig 2.4) and Bayesian analyses generated broadly congruent phylogenetic trees (Fig 2.2 and 2.3). Phylogenetic analyses repeatedly supported the division of three distinct clades; each with strong Bayesian approximated posterior probability values for their monophyly (0.96, 0.86 and 0.99). A further clade appeared but was poorly supported (0.57). Caribbean sequences appear in each of the clades and show high levels of nodal support within these lineages (0.83-1). Within the phylogeny, there is an overall trend for greater support from Bayesian analysis compared with the bootstrap and is likely due to the more conservative nature of bootstrap analyses (Erixon *et al*. 2003). Caribbean sequences are found within lineages containing four of the six African species of *Chlorocebus*: *C. sabaeus*, *C. tantalus*, *C. pygerythrus* and *C. cynosuros*. In one lineage, a Caribbean sequence from Barbados (Carib_BarbBG61) sits alongside multiple *C. tantalus* sequences from Cameroon and Nigeria, with strong support (0.91) between the Caribbean and Nigerian sequences. Caribbean sequences sit within another clade, along two strongly-supported lineages: BARBADOS_060_CARIB sits alongside multiple sequences of *C. cynosuros* (from the DRC region) with strong support (0.99) and ST.KITTS_022_CARIB and ST.KITTS_027_CARIB sit within a cluster of Zimbabwe-originating *C. pygerythrus* sequences, again with a strong (1) level of support. The vast majority of Caribbean sequences (24) fall within one clade (C2), sitting amongst *C. sabaeus* sequences from Nigeria, Senegal, Gambia and Sierra Leone and with *C. pygerythrus* sequences from Zimbabwe (Fig. 2.2). There is once again a strong level of nodal support (0.97) within this lineage-based cluster of sequences. The phylogeny presented five distinct

haplotypes (Table 2.3), with Caribbean sequences featuring in four (Fig. 2.3) of these groupings. The largest of these haplotypes is HAPLO_B009 (19 sequences), with sequences originating from St Kitts (12), Barbados (5) and *C. sabaeus* samples from Sierra Leone (2). Falling within the same lineage but forming a distinct haplotype, HAPLO_B013 (4 sequences) comprised of St Kitts (4) samples only. Within another large, strongly supported clade, haplotypes are again present within two distinct lineages: HAPLO_B006 (2 sequences), which is made up from a Barbados sample and a *C. tantalus* sample from Nigeria and; HAPLO_B002 (3 sequences), which is made up of two *C. cynosuros* samples from Zimbabwe and one sample from St Kitts. Whilst the vast majority of obtained Caribbean sequences are monophyletic, and fall most closely to *C. sabaeus* within the African species, this phylogeny shows that Caribbean *Chlorocebus* monkeys have multiple African origins, from multiple African species.

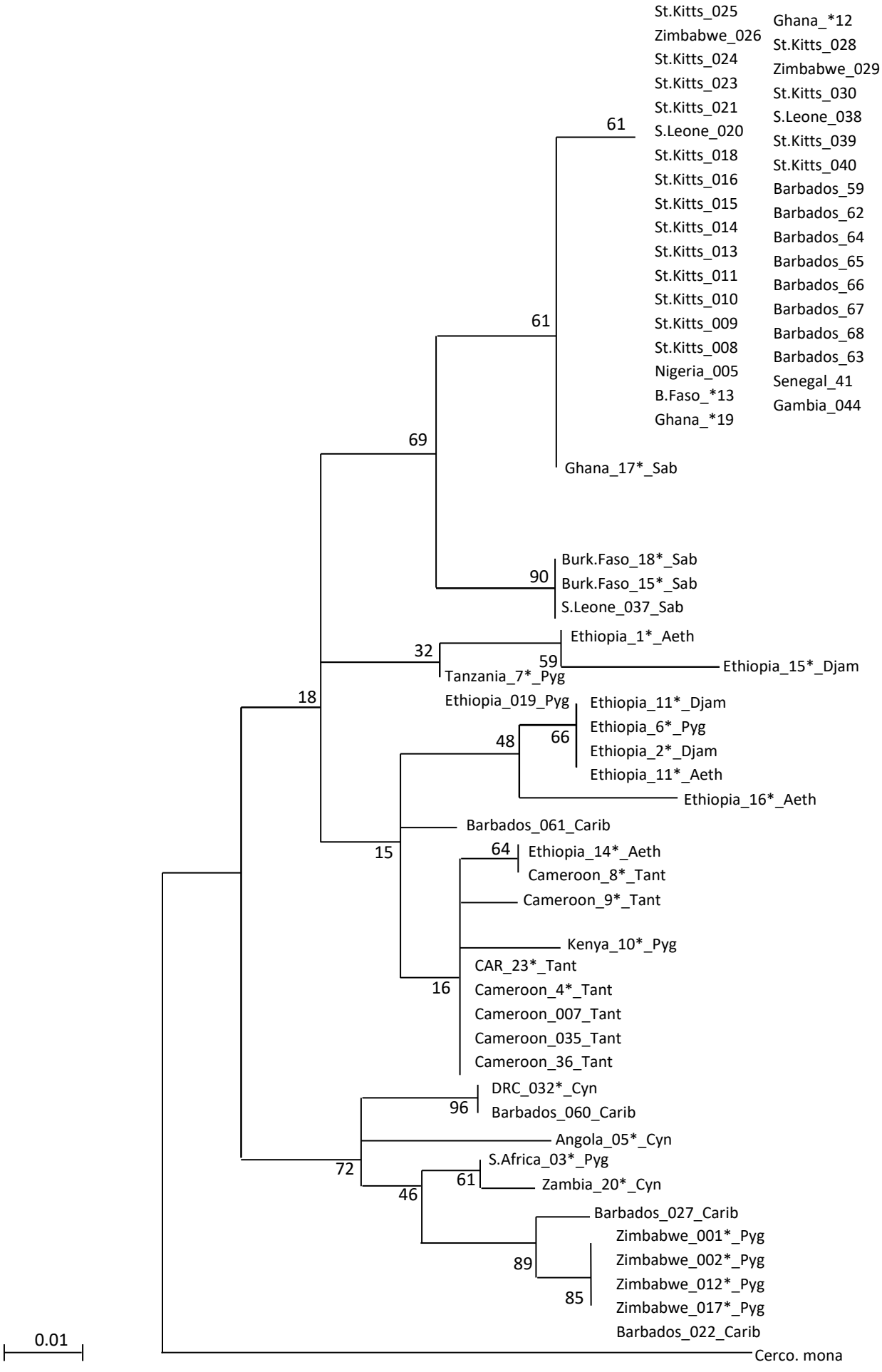

Figure 2.4. Maximum likelihood values, based on 1000 bootstrap replicates.

2.4 Discussion

This study is the first to demonstrate that not only does the Caribbean population of *Chlorocebus* monkeys originate from multiple sources along western Africa but that it stems from at least four different species of African *Chlorocebus* monkey. It is therefore highly unlikely that the establishment of *Chlorocebus* within the Caribbean represents a single colonisation event but instead represents numerous introductions. The phylogeny also highlights that the taxonomy of the *Chlorocebus* genus is both complex and still not fully resolved.

The aim of this study was to see whether (1) the phylogeny supports the current species designations in the African *Chlorocebus* species, (2) the Caribbean populations of *Chlorocebus* represent a single or multiple colonisation event, and (3) if the Caribbean *Chlorocebus* monkeys originate from a single or multiple African source population. In answering (1), looking at the phylogeny appears to initially show a jumbled distribution of genotypes mixed into clades and lineages of varying degrees of strength and support (Fig. 2.2). Within several of the lineages, there is a distinct mixing of African species, with up to three different species being clustered together. Some of the larger haplotypes (e.g. HAPLO_B009) and groupings of distinct African species could be explained by sampling error, with either the original specimen collector or the collection curator incorrectly labelling the specimen species or geographic origin. However, this does not seem to be the case; whilst the phylogenetic tree (Fig. 2.3) does not fully conform to the existing

taxonomic classification of the group largely based on phenotype and behaviour (Kingdon, 1977; Groves, 2001; Cardini *et al*., 2007; Gonedelé *et al*., 2009), it does however support recent molecular research (Haus *et al*. 2013), reflecting geographic regions rather than nominal species. Additionally, although some of the nodal support may at first glance appear weak and where such weak branches could have been collapsed, when the overall pattern of phylogeny was actually compared to the actual geographic distribution of *Chlorocebus* in Africa (Fig. 2.3), they again appear to strongly complement each other. Even with the limited data available, the phylogeny presented here does support recent findings that the taxonomy of *Chlorocebus* monkeys in Africa is both unclear and has possibly been oversimplified in the past. Many of the lineages consist of sequences from areas where, in the wild, hybridisation would not only be possible but probable (as in the clade where Caribbean sequences sit amongst both *C. pygerythrus* and *C. cynosuros*). In one clade, mtDNA from three distinct African species sits within the same grouping, suggesting that the three phenotypes in this area (*C. aethiops*, *C. djamdjamensis* and *C. pygerythrus*) are not only capable of hybridising but actively do so. Based on the strong Bayesian nodal support (0.96) within the phylogeny, this grouping appears to represent a distinct genotypic cluster. In western Africa, the Volta River appears to be an established barrier between *C. tantalus* and *C. sabaeus* and sequences appear in separate (strongly-supported) clades. Further investigation into possible samples from the Togo-Ghana border region where hybridisation might occur would help to confirm this finding. In this phylogeny, sample data from both Burkina Faso and Ghana were sequenced for the first time and

showed a distinct split within sequences from both countries. Some samples from Burkina Faso and Ghana sit close to sequences from Sierra Leone and the Sene-Gambia region, whereas others (with strong support) sit alongside individuals from Nigeria in different lineages within different clades. Whilst all of these samples were labelled *C. tantalus*, they appear in this phylogeny to belong to two distinct genotypes. Whilst the phylogeny supports recent previous findings (Haus *et al*. 2013), there are still areas that remain ambiguous, such as a clearer resolution of haplotype HAPL_B009. Whereas past research has sequenced the complete mitochondrial Cyt *b* gene, the work described here mostly used sequences that were 259 bp long. Nonetheless, some sequences were of the complete Cyt *b* region, but the most important sequences obtained in this study were shorter mainly due to the fragmentary nature of DNA in aged museum specimens, thus making an overall trimming of the sequences necessary. Many of these important sequences were either Caribbean samples from museums or museum samples representing African *Chlorocebus* from areas which had not been sequenced before this study; such as Ghana and Burkina Faso (Haus *et al*. 2013). Potentially, if sequence lengths had been longer, then either some of the lineage branches could have stronger support and some of haplotypes could have been resolved. Further study using either much longer mtDNA sequences or nDNA would help to further understand the complex nature of this African *Chlorocebus* phylogeny. Through a combination of influencing factors, Bayesian posterior probabilities are often “excessively high” (Cummings *et al*. 2003) and the relationship between posterior probability and bootstrap support is complex and still not entirely understood. Often, there is a

discrepancy in terms of assessing nodal confidence (Alfaro *et al*. 2003) in relation to the results generated. Because, after assessment from multiple simulated analyses, it is generally accepted that Bayesian posterior probabilities are a less biased predictor of phylogenetic accuracy (Alfaro *et al.* 2003), the results generated from the Bayesian posterior probabilities generated in these analyses described here are considered to be more 'reliable' in this instance.

In addressing (2); whether the Caribbean populations of *Chlorocebus* represent a single or multiple colonisation event, and (3) if the Caribbean *Chlorocebus* monkeys originate from a single or multiple African source population, it is clear that Caribbean *Chlorocebus* monkeys are split across several clades within the phylogeny. Across these clades, Caribbean *Chlorocebus* monkeys appear alongside *C. sabaeus*, *C. tantalus*, *C. pygerythrus* and *C. cynosuros*. For each of these species, at least part of their range is coastal western or southern Africa. This distribution would have provided the means for African *Chlorocebus* (from multiple species) to have been transported from Africa to the Caribbean. Within the phylogeny, no Caribbean sequences are found to be closely associated with either *C. aethiops* or *C. djamdjamensis*. With neither of these two African species being found either in western Africa or in close proximity to ports used for the transport of enslaved Africans across the Atlantic (Eltis and Richardson, 2010), their lack of association is both unsurprising and adds support to the idea that Caribbean *Chlorocebus* originated from multiple points (covering several species) from western and southern Africa. The phylogeny provides

strong Bayesian and bootstrap support for the inclusion of Caribbean *Chlorocebus* sequences amongst several distinct lineages, within three separate clades, covering four different African species of *Chlorocebus*. This phylogeny therefore demonstrates that (2) the Caribbean *Chlorocebus* monkeys originate from at least four different sources in Africa (covering the four source species) and that (3) due to the fact that the Caribbean *Chlorocebus* population has been shown to have such a diverse source history, the Caribbean monkeys are highly unlikely to originate from one colonisation event and are instead far more likely to have resulted from multiple introductions. These results indicate that the Barbados population appears to have maintained strong association with four distinct phylogenetic sources (*C. sabaeus, C. tantalus, C. pygerythrus* and *C. cynosuros*), corresponding to existing and distinct phenotypes (and species) in Africa, implying that the current population of *Chlorocebus* monkeys on Barbados originated from at least four (and probably many more) introduction events from different source species and populations of *Chlorocebus* in Africa. The St. Kitts monkeys appear to originate from two main African source populations from Sierra Leone/Ghana-originating *C. sabaeus* and from *C. pygerythrus* from the region around Zimbabwe and Zambia. Again, this Caribbean population also originates from multiple distinct source populations in Africa, and have most likely arisen from multiple colonisations. As some St Kitts monkeys are nested with some Barbados monkeys (along with others from Sierra Leone and Ghana), it is possible that they were introduced either at the same time (from the same introduction event) or sequentially, from one Caribbean island to the other.

Many of the phylogenetic relationships among clades remain largely unresolved, due to low statistical support in several of the clades, using a Bayesian approach. Additionally, the fact that there are several examples of discrepancies between the observed phenotypes and the mtDNA (discounting the influence of short sequence lengths) indicate that hybridization may be influential within this genus (Haus *et al*., 2013), as it is throughout many of the cercopithecines (Groves, 2001; Detwiler *et al.* 2005; Chatterjee *et al*. 2009). An example of such discrepancy can be seen across the African *C. pygerythrus* samples, where although it is a widespread species based largely on phenotype (Kingdon, 1997), the molecular phylogeny shows that many *C. pygerythrus* individuals (across various parts of its range) share closer genetic affiliations with other species (i.e. *C. djamdjamensis*, *C. aethiops* and *C. cynosuros*) than it does with its own conspecifics. Alternatively, this disparity may instead be due to the fact that the *Chlorocebus* phenotypes are plastic and that they do not show strong correlations with designated species lineages but instead with geographical associations. If this is the case, then the discrepancies seen may simply reflect that the samples have come from broad geographic regions. Because of this observed phylogenetic discordance and because there is often a more parsimonious link between the observed phenotype and the nDNA (Lanfear *et al*. 2010; Suárez-Díaz, 2014), further investigation using nDNA may resolve several unanswered aspects of *Chlorocebus* molecular taxonomy. Also, by accepting Bayesian posteriors as strong evidence for Caribbean *Chlorocebus* monkeys having originated from multiple African source species (or genotypically-distinct populations), then it must be noted that along some branches of the phylogeny (Fig. 2.3), some

southern African samples of *C. pygerythrus* are grouped with West African *C. sabaeus* samples (see haplotype HAPLO_B009). Potentially, this is due to incomplete taxon sampling of *C. cynosuros,* as a total of five specimens were available. As incomplete sampling such as this has been linked with such disparate results in phylogenies (Rosenber and Kumar, 2001; Ryber and Matheny, 2011), future studies should include a larger sample set from this taxon, in order to assess whether this finding is as a result of incomplete sampling or because of a phenomenon within this grouping. This result does not affect the position of Caribbean samples (either with each other or with other African taxa), as it was possible to more comprehensively sample and represent other specimen groups.

These results provide strong support for the idea that Caribbean *Chlorocebus* monkeys have originated from multiple African source populations, and that approximately 400 years after they were first introduced, individuals descended from different source populations have maintained distinct phylogenetic profiles within the same islands. This directly contrasts with previous research, where the general consensus was that Caribbean monkeys are all synonymous with *C. sabaeus* from the Sene-Gambia region of Africa (van der Kuyl et al. 1996; Pandrea et al. 2006; Haus et al. 2013).

As is seen across multiple taxa, cercopithecines utilise visual signals in order to act as isolating barriers to prevent interbreeding of populations through a role in species recognition (Kingdon, 1980; Allen *et al*. 2014). As many of these guenons are sympatric with one another in Africa, they have evolved distinctive facial colouration and patterning in order to distinguish conspecifics

from heterospecifics, often with even young lineages showing significant levels of phenotypic differences. Species of African *Chlorocebus* have been shown to have significantly different faces from another (Allen *et al*. 2014). If the founder population or populations on Caribbean islands were from different source populations in Africa, then it is possible that facial differences in terms of markings and colouration acted (and continue to act) as means of character displacement and serve to act as a reproductive isolation barrier within each of the Caribbean islands, effectively segregating specific groups into smaller sub-units and maintaining the distinct African phylogenetic lineages. Future investigation of the Caribbean *Chlorocebus* nDNA would help ascertain if these distinct lineages are simply a result of non-recombining mtDNA lineages not going locally extinct or whether specific facial markings and colouration between and across Caribbean populations are acting in some capacity as isolating mechanisms.

In support of recent findings (Haus *et al*. 2013), whilst this phylogeny found that the mtDNA diversity of African *Chlorocebus* monkeys is in discordance with the traditionally accepted taxonomy of the genus, the incomplete sequencing of the mtDNA genome means that many of the observed discrepancies cannot be resolved. This finding, in addition to the suspected finding of probable hybridisation means that the definitive phylogeny of the African *Chlorocebus* group cannot be resolved without the use of nDNA phylogenetics. However, this study did find that there are multiple instances where mtDNA data do not fit with existing phenotypic assignment within the genus in Africa.

2.5 Conclusion

This study proposes that wild-living Caribbean *Chlorocebus* monkeys stem from numerous western African sources, showing clear molecular links from three different species of African *Chlorocebus* monkey. The establishment of the Caribbean *Chlorocebus* is not likely to have come from a single colonisation event associated with the trans-Atlantic slave trade but instead represents numerous introductions across a time frame of several centuries (McGuire, 1974; Denham, 1987). The phylogeny also highlights that the taxonomy of the *Chlorocebus* genus is both complex and still not fully resolved. This analysis strongly suggests that a large amount of hybridization has occurred across African *Chlorocebus* species and that, from a molecular viewpoint, the differentiation and designation of these species may not be appropriate. Despite this existing molecular discordance in African *Chlorocebus,* the results here show that the populations of African *Chlorocebus* monkeys represent hybridised groups, stemming from clearly separated African source populations, corresponding with major historical ports associated with the trade in enslaved Africans. Caribbean monkeys do not yet show any clear signs of molecular divergence from their African ancestral populations (at a mtDNA level) but instead retain these clear African associations.

Whilst this phylogeny does not fully support the traditional taxonomy of African *Chlorocebus* designations, it does largely agree with recent mtDNA findings,

reflecting the need for a better understanding of the *Chlorocebus* clade as a whole, based on actual geographical distribution rather than nominal species designation. When looking at the heritage of the Caribbean *Chlorocebus* monkeys, it appears that they were introduced into the Caribbean from at least four locations in Africa, that they originate from four species of African *Chlorocebus* and whilst there is no direct evidence here, it seems highly unlikely that the Caribbean populations resulted from only a single colonization and instead are the product of multiple colonization events. *Chlorocebus* monkeys are used in the Caribbean to investigate and develop AIDS research through looking at viral replication and immune responses in natural hosts infected with SIV (Pandrea *et al*. 2006), with the research being based on the assumption (from a limited dataset) that all Caribbean *Chlorocebus* monkeys are *Chlorocebus sabaeus.* Each *Chlorocebus* species has its own distinct SIV subtype in the wild, yet laboratory animals are all infected with the *C. sabaeus*-specific SIVagm.sab subtype and are then monitored and treated according to this precept. In light of this and the potential for inaccurate results from such biomedical research, further investigation is needed into this area, to wholly resolve the complete phylogeny of Caribbean *Chlorocebus* monkeys, using nDNA analyses.

The traditional notion that Caribbean *Chlorocebus* originate from a Senegal-based *Chlorocebus sabaeus* ancestor has been disproved by looking more closely at the genetics of the introduced populations of *Chlorocebus* from the Caribbean. With several distinct source populations in Africa, it is likely that

there were multiple introductions from multiple sources, from numerous populations, representing four distinct African genotypes.

In terms of the African *Chlorocebus* groupings, no taxonomic changes are considered here. However, when looking at the Caribbean *Chlorocebus* populations, whilst the phylogenies are not fully resolved (and require full mtDNA or nDNA genomic studies), this study has revealed that rather than all being exclusively descended from African *C. sabaeus* animals, these populations are instead comprised of animals from numerous and distinct African lineages, from along much of coastal western Africa.

Chapter 3

Shape analysis of Caribbean and African *Chlorocebus* crania using 3D geometric morphometrics methods to investigate possible island divergence.

3.1 Introduction

Although the cranial base is not always the most reliable part of the skull in reflecting phylogenetic history (Bjarnason *et al*. 2015), the external morphological features of primate cranial bones are frequently used to explore a broad range of topics, from speciation (Burness *et al*. 2001; Ackermann *et al*. 2014; Berger *et al*. 2015; Solórzano-García *et al*. 2015) and taxonomy (Balzeau *et al*. 2006; Baab *et al*. 2008; Delmore *et al*. 2013; Parker *et al*. 2014; Solórzano-García *et al*. 2015), to systematics (Chapman, 1983; Baab *et al*. 2009; Fjeldså *et al*. 2012; Ackermann *et al*. 2014) and primate biogeography (Chapman, 1983; Schillaci *et al*. 2009; Bettridge *et al*. 2012; Allen *et al*. 2013) in both extant non-human primates and fossil hominins. For example, at latitudes between 8° S and 13° N, cranial length in the crab-eating macaque (*Macaca fascicularis*) increases with latitude and decreasing temperatures on both sides of the Equator (Fooden and Albrecht, 1993; Cardini *et al*. 2007). However, despite primate biogeography being the source of great research interest (Alfaro *et al*. 2015), taxonomic distinctions in primates may often rest on seemingly qualitative morphological features such as pelage colouring and patterning (Kamilar *et al*. 2011; Morales-Jiminez *et al*. 2015), potentially confounding results. Instead of using more qualitative characters such as pelage and body coloration, the use of cranial morphology provides a rigorous basis for primate systematic descriptions, phylogeny and functional anatomy (Cardini *et al*. 2008; Fleagle *et al*. 2010), where geometric morphometric methods can be used for the quantitative analysis of form variation (Frost *et al*. 2003;

Goswami, 2006; Cardini *et al*. 2007; Baab *et al*. 2008; Cardini *et al*. 2008; Elton *et al*. 2010; Fleagle *et al*. 2010; Gilbert, 2010; Parr *et al*. 2011). Much of the existing research in primate cranial morphology or evolution traditionally focused on specific cranial modules such as the auditory or olfactory regions or the basicranium (Fleagle *et al*. 2010), where cranial overviews were often descriptive and qualitative. Such endochondrally ossifying parts of the cranium reflect phylogeny well because of their early ossification, lack of impact by the strains of mastication and their hypothesized limited exposure to environmental effects (Roseman *et al*. 2010). Of these regions, the basicranium has been argued to contain a very strong phylogenetic signal in previous analyses of primate cranial morphology (Gilbert, 2010). As an anatomical region, the basicranium is an important integration centre for many functional systems. Brain size, brain shape, cranial nerve supply, mastication, hearing, blood supply, posture and locomotion are all said to influence the basicranium anatomy (MacPhee and Cartmill, 1986; Strait, 2001; Strait and Grine, 2004; Lockwood *et al*. 2005; Gilbert, 2010) and due to the functional importance of these systems, basicranial morphology is likely to be both highly heritable and to show a lower incidence of homoplasy. The cranial base is also said to constrain cranial evolution because of its numerous functional and developmental roles as the interface between the cranial vault and face and the remainder of the body (Roseman *et al*. 2010). Traditionally, many studies in primate cranial morphology focused on scaling patterns within and among species, with comparatively few looking at differences in cranial

shape, other than scaling patterns (Frost *et al*. 2003). When exploring morphological divergence within a group of closely related animals, it is important to consider allometric influences. The work described here is concerned only with adult specimens however, so that static allometry is the type most relevant to this study, where size-related shape differences in individuals of similar age within species are prominent (Klingenberg, 1996; Elton *et al*. 2010). In many studies involving morphometrics, there exists an assumption that where available, previously existing phylogenetic frameworks available for the study taxa are correct (Cardini *et al*. 2008) and that any subsequent morphological analysis works within these parameters. However, morphological studies have been used to aid in the taxonomic clarification where existing phylogenetic relationships are unclear within a primate taxon (Gilbert, 2010). Across the primates, there are numerous examples of taxonomic uncertainty and taxa in need of clarification and revision, with maybe none more so than the green monkeys (*Chlorocebus* spp.) of Africa (Groves, 2001; Bi *et al*. 2009). Whilst morphometrics have been used to study the African cercopithecine primates (Roseman *et al*. 2010) and *Chlorocebus* has been individually assessed and reviewed (Cardini *et al*. 2007; Elton *et al*. 2010), the status of *Chlorocebus* remains unclear and contested.

Chlorocebus monkeys (widely known as either green monkeys or vervets) are among the most widespread of the African primates, inhabiting a wide swathe of sub-Saharan Africa (Kingdon, 1997; Bi *et al*. 2009). They are

found across the continent from north-west Senegal to Ethiopia, Djibouti and Somalia, and as far south as South Africa. *Chlorocebus* monkeys live in a wide variety of habitats, showing preference for savannah habitats and savannah forest mosaics. There is a relative paucity of research in the taxonomic signals in *Chlorocebus* craniodental characters, apart from a few notable exceptions (Cardini *et al*. 2007; Elton *et al*. 2010), where skull variation was observed to follow variation across a mostly longitudinal cline, largely reflecting size variation. In a study that classed the six African *Chlorocebus* species as six subspecies as part of a monophyletic group (Elton *et al*. 2010), 86 three-dimensional landmarks were taken from a sample of *Chlorocebus* monkeys, where morphometric analysis showed cranial differences were most apparent over a west-to-east distribution across the described subspecies and with central subspecies showing intermediate differences between the two extremes. One of the main observations was that travelling along a west to east cline, a shortening of the face and relative expansion of the neurocranium was apparent. Assessing patterns of such interpopulational variation is crucial to clarifying evolutionary divergence (Elton *et al*. 2010), with such analyses furthering an understanding of the biological responses to past and present environments, the associated environmental processes and drivers of speciation. Despite the analysis of phenotypic character variation along a geographic and temporal scale being a reliable means for evaluating how traits evolve at both intra- and interspecific levels (Bozinovic *et al*. 2011), the taxonomic status and phylogenetic relationships of *Chlorocebus* remain

unclear and the taxonomy of the entire genus is still in urgent need of revision (Bi *et al*. 2009). Previous findings showed highly significant variation was observed following clinal variation in size and shape (Cardini *et al*. 2007), with similarities being apparent between males and females. Size variation was present, accounting for approximately 40% of the observed differences, with results reflecting longitudinal rather than latitudinal clines, especially across a western to eastern pattern. Additionally, a strong spatial and environmental basis to variations in African *Chlorocebus* was observed, with levels of rainfall being an important contributing indicator for variation in cranial size and shape. Reproductive isolation can often translate into subtle yet highly discriminating differences in hard tissue, even in very closely-related species with short divergence times (Elton *et al*. 2010), making *Chlorocebus* monkeys an ideal group in which to study the early stages of evolutionary divergence (Elton *et al*. 2010).

Since the inception of the field of biogeography (Wallace, 1895), islands have been recognised as being of great importance in providing numerous opportunities for illuminating evolutionary and ecological processes (Emerson and Kolm, 2005; Wilmé *et al.* 2006; Whittaker *et al.* 2008; Brown *et al.* 2013). Insular ecosystems represent cradles of biodiversity (Fjeldså *et al.* 2012) and are often referred to as being 'natural laboratories' (Brown *et al.* 2013; Brace *et al.* 2015), as evolutionary processes are often more readily observed over shorter timeframes. Introduced insular populations

are more likely to follow rapidly divergent evolutionary trajectories with subsequent speciation (Millien, 2006; Schillaci *et al.* 2009) and distinctive morphological features in vertebrates can become apparent after as little as 20 generations (Stuart *et al.* 2014). While the evolutionary mechanisms underlying successful invasions are not well understood (Purcell *et al.* 2012), such rapid insular speciation is typically thought to result from these populations experiencing different selective pressures from their mainland congeners (Schillaci *et al.* 2009). With insular populations very often undergoing evolutionary changes as a result of the specialised and unique characteristics of island ecosystems (Lomolino, 2005), the study of such introductions is of huge importance to the work of evolutionary biologists, ecologists and biogeographers. Compared to mainland populations, insular animals often have limited resources, fewer predators and increased intraspecific competition (Lomolino, 2005; Donlan and Wilcox, 2008; Schillaci *et al.* 2009), with resource competition having been the interaction suggested most often as the main source of such insular divergent selection (Stuart *et al.* 2014).

The use of morphometrics can significantly help elucidate the processes associated with adaptations to insular ecosystems (Lomolino, 2005) and can be used to investigate well-documented, yet complex historical insular mammalian introductions (Barun *et al.* 2013). Compared to their mainland counterparts, insular-living populations can show drastic changes in size, morphology and even possibly longevity (Foster, 1964). Much research has

investigated the phenomenon termed the 'island rule', where small-bodied species tend to evolve towards gigantism on islands, but larger-bodied species tend towards dwarfism on islands (Foster, 1964; Van Valen, 1973; Meiri *et al.* 2006; Bromham and Cardillo, 2007). Island dwarfism has been recorded in carnivores, lagomorphs and artiodactyls and gigantism in rodents and marsupials (Lomolino, 2005). More specifically, cranial morphometrics can be a powerful tool in investigating insular systematics and taxonomy (Turvey *et al.* 2006; Carden *et al.* 2012) but while introduced insular populations of some mammals such as the Soemmerring's gazelle (*Nanger soemmerringii*) have markedly different cranial shapes (Chiozzi *et al.* 2014) even a limited dataset (of cranial landmarks) can be used to resolve areas of insular systematics and taxonomy (Schillaci, 2010). Whilst there does appear to be a paucity of primate-based insular biogeography research (Lomolino, 2005), islands are ideal for studying primate taxonomy and systematics (Nijman and Meijaard, 2008). In terms of changes in size as a response to island living, there is some disagreement within the existing data, with some suggesting that insular primate populations do undergo predictable shifts in body size (Bromham and Cardillo, 2007) even on islands not very distant from larger landmasses and over relatively short time-scales, others report that primates do not clearly conform to the island rule in terms of changes in body mass (Schillaci *et al.* 2009). When looking at changes in cranial shape however, island primates have often been shown to demonstrate marked cranial morphological variation across island archipelago ecosystems (Fooden and Albrecht, 1993; Schillaci *et al.* 2009;

Rosenberger *et al.* 2010; Montgomery and Mundy, 2013) and even a very limited dataset of cranial landmarks can be used to study insular biogeography in primates (Schillaci, 2010), where small morphological differences such as canine or zygomatic width has been used to separate some primate species (Mercês *et al.* 2015). In an insular ecosystem, the use of morphometrics can enable primate taxa which have been historically very taxonomically muddled to be resolved using the phylogenetic species concept, where consistent, fixed morphological differences across geographically defined groups are identified (Biswas *et al.* 2011; Mercês *et al.* 2015).

Within the Caribbean, there is strong evidence that human activities have drastically altered the existing ecology of numerous species (Giovas *et al.* 2012), with such anthropogenic impacts being most marked during the historical period. In addition to this, West Indian land mammals have suffered the most severe extinctions of any prehistorical fauna (Turvey *et al.* 2006), making the region of particular importance in terms of understanding not only insular speciation and systematics but in conservation action plans also (Rosenberger *et al.* 2010; Hansford *et al.* 2012; Brace *et al.* 2015). African monkeys (*Chlorocebus* spp.) were introduced from western Africa to the Caribbean between the 17-19th centuries and now form populations on the islands of Nevis, St Kitts and Barbados. Whilst the history of the introduction and establishment of these monkeys is relatively well documented (Sade and Hildrech, 1965; Denham,

1987; Van der Kuyl *et al*. 1996), they were not observed in a rigorous sense until Ashton and Zuckerman (1950) looked at cranial and dental differences between monkeys from St Kitts and green monkeys (*Chlorocebus sabaeus*) from Senegambia region in western Africa. These early studies showed that the morphological divergence between the St Kitts and African *C. sabaeus* was similar to that observed between species of the genus (when *Chlorocebus* was still subsumed within the *Cercopithecus* taxon) and larger than that between many existing African *Cercopithecine* subspecies (Ashton and Zuckerman, 1950; Ashton and Zuckerman, 1951*a*; Ashton and Zuckerman, 1951*b*; Ashton *et al*. 1979). In overall size, both the dental and cranial morphology are larger in the St Kitts monkeys than those from an African origin (Ashton and Zuckerman, 1950), especially in the buccolingual region and in the breadth of the cranium. It was also shown that certain meristic features in the St Kitts crania such as the number and positioning of teeth were significantly more variable than those from West African crania (Ashton and Zuckerman, 1951*a*) and that variance in the cheek teeth, calvarium and facial region of the skull is less in the Caribbean monkeys than in the West African *C. sabaeus* monkeys (Ashton and Zuckerman, 1951*b*). Variance due to bilateral dissimilarities was greater in the island monkeys than in those measured from Africa (Ashton *et al*. 1979). Such asymmetry is thought to reflect a lower degree of developmental stability in the island population. When looking at dental morphology, the differences between St Kitts monkeys and African *C. sabaeus* was at a similar level to that observed between *C. sabaeus* and

some African guenon species (Ashton *et al*. 1979) yet less than that between *C. sabaeus* and another *Chlorocebus* species (*C. aethiops*). However, much of these earlier cranial and dental analyses of the Caribbean *Chlorocebus* monkeys used univariate techniques (Ashton and Zuckerman, 1950; Ashton and Zuckerman, 1951*a*; Ashton and Zuckerman, 1951*b*). Some of these earlier data were later reanalysed through more rigorous multivariate analyses (Ashton *et al*. 1979). Because of these earlier techniques, many of these measurements were broadly averaged and precise shape differences were not discernable. Additionally, only a relatively small sample size was available due to the common occurrence of post mortem trauma on the skulls and from those which were measured, it was common that an equal number of landmarks could not be measured across individual crania, which was not accounted for in analyses. Throughout all these analyses, only a maximum of 24 dental and 49 cranial landmarks were used for analyses, potentially missing out small cranial differences. Overall, both the teeth and skulls of St Kitts monkeys were bigger and less variable than those of the West African *C. sabaeus* monkeys.

This study aims to use *Chlorocebus* as a model for interspecific variation across island taxa, assessing any observed differences and variation through hard tissue cranial morphology. It also aims to use morphometrics including the basicranium, maxi-facial and temporal region to provide a quantitative analysis of cranial shape among the African *Chlorocebus* taxon

to 1) identify major patterns in cranial shape across the group; 2) identify whether any cranial morphometric divisions correspond with existing current *Chlorocebus* taxonomy and; 3) see whether the findings help resolve the *Chlorocebus* taxonomic ambiguity. An assessment is also made of the three populations of Caribbean *Chlorocebus* monkeys, using morphometrics to see whether there are quantifiable differences 4) between these three insular populations and; 5) if there is any correlation between these Caribbean monkeys and any of the African *Chlorocebus* taxa. This study focuses on shape variation and differences in the crania.

3.2 Methods

3.2.1 Samples

A total of 114 *Chlorocebus* crania (Table 3.1) were digitized between 2011 and 2013 at museums in the UK and from the three Caribbean islands. In addition to specimens from each of the three Caribbean island populations, this sample includes three of the six recognised African species; *C. sabaeus*, *C. tantalus* and *C. cynosuros*, representing those species found along the western African seaboard. Of this scanned total, 114 specimens were used, with 77 Caribbean and 37 African specimens preserving all of the landmarks used here. Only complete specimens were used in these analyses. While the African sample is not evenly distributed geographically, it does cover nearly the entire range of all the included species. The sample localities of all specimens included in these analyses (when known) are included. Within the sample used, 90 specimens were male and 24 were female. Although it cannot be quantified, this apparent strong male bias is likely due to a bias for hunting males (pers. obs.), partly due to a conceived idea that killing males has a greater impact on population control. The maturity of each specimen was assessed on the basis of full dental eruption of the canines and third molars (Cardini *et al*. 2007). Specimens were only digitized when accompanying data reliably detailed its sex and geographic origin. In many instances, the species was recorded but due to changing and updating taxonomic status, each designation was confirmed through

Table 3.1. Cranial samples from Caribbean and African specimens, where 'RCS' refers to the Royal College of Surgeons (UK), 'NHM' to the Natural History Museum (UK) and * denotes a Caribbean population of *Chlorocebus*.

Species	Accession	Location	Museum	Sex
*Carib.**	RCSOMA72.615	St Kitts	RCS	M
*Carib. **	RCSOMA72.618	St Kitts	RCS	M
*Carib. **	RCSOMA72.634	St Kitts	RCS	M
*Carib. **	RCSOMA72.638	St Kitts	RCS	M
*Carib. **	RCSOMA72.642	St Kitts	RCS	M
*Carib. **	RCSOMA72.643	St Kitts	RCS	F
*Carib. **	RCSOMA72.645	St Kitts	RCS	F
*Carib. **	RCSOMA72.647	St Kitts	RCS	F
*Carib. **	RCSOMA72.648	St Kitts	RCS	M
*Carib. **	RCSOMA72.652	St Kitts	RCS	M
*Carib. **	RCSOMA72.653	St Kitts	RCS	M
*Carib. **	RCSOMA72.655	St Kitts	RCS	M
*Carib. **	RCSOMA72.659	St Kitts	RCS	M
*Carib. **	RCSOMA72.663	St Kitts	RCS	M
*Carib. **	RCSOMA72.665	St Kitts	RCS	M
*Carib. **	RCSOMA72.667	St Kitts	RCS	M
*Carib. **	RCSOMA72.668	St Kitts	RCS	M
*Carib. **	RCSOMA72.673	St Kitts	RCS	M
*Carib. **	RCSOMA72.678	St Kitts	RCS	F
*Carib. **	RCSOMA72.681	St Kitts	RCS	M
*Carib. **	RCSOMA72.683	St Kitts	RCS	F
*Carib. **	RCSOMA72.6695	St Kitts	RCS	M
*Carib. **	RCSOMA72.6698	St Kitts	RCS	M
*Carib. **	Nev002	Nevis	Field	M
*Carib. **	Nev003	Nevis	Field	M
*Carib. **	Nev004	Nevis	Field	M
*Carib. **	Nev005	Nevis	Field	M
*Carib. **	Nev006	Nevis	Field	M
*Carib. **	Nev007	Nevis	Field	F
*Carib. **	Nv13AF01	Nevis	Field	F
*Carib. **	Nv13AF02	Nevis	Field	F
*Carib. **	Nv13AF03	Nevis	Field	F
*Carib. **	Nv13AF04	Nevis	Field	F
*Carib. **	Nv13AF05	Nevis	Field	F
*Carib. **	Nv13AF06	Nevis	Field	F
*Carib. **	Nv13AM01	Nevis	Field	M
*Carib. **	Nv13AM02	Nevis	Field	M
*Carib. **	Nv13AM03	Nevis	Field	M
*Carib. **	Nv13AM04	Nevis	Field	M
*Carib. **	Nv13AM05	Nevis	Field	M
*Carib. **	Nv13AM06	Nevis	Field	M
*Carib. **	Nv13AM07	Nevis	Field	M
*Carib. **	Nv13AM08	Nevis	Field	M
*Carib. **	Nv13AM09	Nevis	Field	M
*Carib. **	Nv13AM10	Nevis	Field	M
*Carib. **	Nv13AM11	Nevis	Field	M
*Carib. **	Nv13AM12	Nevis	Field	M
*Carib. **	Nv13AM13	Nevis	Field	M
*Carib. **	Nv13AM14	Nevis	Field	M
*Carib. **	Nv13AM15	Nevis	Field	M
*Carib. **	BDS13AFC3885	Barbados	Field	F
*Carib. **	BDS13AFC3888	Barbados	Field	F

Table 3.1. *contd.*

*Carib. **	BDS13AFC3936	Barbados	Field	F
*Carib. **	BDS13AFC4009	Barbados	Field	F
*Carib. **	BDS13AFC4099	Barbados	Field	F
*Carib. **	BDS13AFC4803	Barbados	Field	F
*Carib. **	BDS13AFB6640	Barbados	Field	F
*Carib. **	BDS13AFC2524	Barbados	Field	F
*Carib. **	BDS13AFC3913	Barbados	Field	F
*Carib. **	BDS13AFC4045	Barbados	Field	F
*Carib. **	BDS13AMA3967	Barbados	Field	M
*Carib. **	BDS13AMA5460	Barbados	Field	M
*Carib. **	BDS13AMB0307	Barbados	Field	M
*Carib. **	BDS13AMB1059	Barbados	Field	M
*Carib. **	BDS13AMB2250	Barbados	Field	M
*Carib. **	BDS13AMB4436	Barbados	Field	M
*Carib. **	BDS13AMB4460	Barbados	Field	M
*Carib. **	BDS13AMB6875	Barbados	Field	M
*Carib. **	BDS13AMB7026	Barbados	Field	M
*Carib. **	BDS13AMB7062	Barbados	Field	M
*Carib. **	BDS13AMB9025	Barbados	Field	M
*Carib. **	BDS13AMB9125	Barbados	Field	M
*Carib. **	BDS13AMB9799	Barbados	Field	M
*Carib. **	BDS13AMB9959	Barbados	Field	M
*Carib. **	BDS13AMB9974	Barbados	Field	M
*Carib. **	BDS13AMC3730	Barbados	Field	M
*Carib. **	BDS13AMC3894	Barbados	Field	M
*Carib. **	BDS13AMC4068	Barbados	Field	M
*Carib. **	BDS13AMC4217	Barbados	Field	M
*Carib. **	BDS13AMC4306	Barbados	Field	M
C. sabaeus	RCSOG53.2	S. Leone	RCS	M
C. sabaeus	20.7.10.4	S. Leone	NHM	M
C. sabaeus	20.7.10.5	S. Leone	NHM	M
C. sabaeus	20.7.10.7	S. Leone	NHM	M
C. sabaeus	20.7.10.8	S. Leone	NHM	M
C. sabaeus	20.7.10.10	S. Leone	NHM	M
C. sabaeus	20.7.10.13	S. Leone	NHM	M
C. sabaeus	46.838	S. Leone	NHM	M
C. sabaeus	54.922	S. Leone	NHM	M
C. sabaeus	82.207	Gambia	NHM	F
C. sabaeus	82.625	Gambia	NHM	F
C. sabaeus	82.629	Gambia	NHM	M
C. sabaeus	82.630	Gambia	NHM	M
C. sabaeus	82.631	Gambia	NHM	M
C. sabaeus	81.734	Gambia	NHM	M
C. sabaeus	56.264	Ghana	NHM	F
C. sabaeus	56.266	Ghana	NHM	M
C. sabaeus	71.2356	Ghana	NHM	F
C. sabaeus	11.6.10.3	G. Bissau	NHM	M
C. sabaeus	09.11.2.1	Senegal	NHM	F
C. tantalus	RCSOMA71.4	Cameroon	RCS	M
C. tantalus	RCSOMA71.1	Nigeria	RCS	M
C. tantalus	RCSOMA71.2	Nigeria	RCS	M
C. tantalus	RCSOMA71.3	Nigeria	RCS	M
C. tantalus	RCSOMAG99.2	Nigeria	RCS	M
C. tantalus	69.1152	Cameroon	NHM	M
C. tantalus	7.7.8.3	Nigeria	NHM	M
C. cynosuros	RCSOMA74.25	Zimbabwe	RCS	M
C. cynosuros	RCSOMA74.22	Zimbabwe	RCS	M
C. cynosuros	RCSOMA74.23	Zimbabwe	RCS	M
C. cynosuros	RCSOMA74.26	Zimbabwe	RCS	M
C. cynosuros	26.11.1.19	DRC	NHM	M
C. cynosuros	21.7.18.2	Zambia	NHM	M
C. cynosuros	69.10.9.13	Zambia	NHM	M

the geographic origin of the specimen. Field samples collected from the Caribbean (Nevis and Barbados) were taken from freshly killed animals, which had been culled as part of ongoing, government-led programmes. All research on this project complied with ethical protocols and procedures set out by University College London (UK), the Zoological Society of London (UK), the Barbados Primate Research Centre and Wildlife Reserve, and the Nevis Ministry of Agriculture, Marine Resources and Cooperatives. These freshly killed specimens were skinned and stripped and then cleaned using warm water maceration for a minimum of four hours using a weak sodium perborate solution. Once the specimens were cleaned, crania were dried and exposed to sunlight over a 24 hour period. Throughout this cleaning process, high levels of safety precautions were taken to remove the potential of zoonotic disease transmission. Latex gloves were worn whenever any primate soft tissue material was handled. Additionally, Kevlar® undergloves (Agar Scientific, UK), cut-proof wrist supports (S. Murray & Co, UK) and bullhead scalpel blades (Swann-Morton®, UK) were used to prevent cuts. Protective clothing, safety glasses and masks were always worn when handling fresh primate material.

3.2.2.Digitization

All samples were digitally scanned using a Next Engine® laser scanner, which has a scanning accuracy of 0.38 mm, using ScanStudio™ HD software. All specimens were scanned with the use of an armature turntable attachment to enable scanning from multiple planes, with the

turntable being positioned parallel with the scanner. For each specimen, scans were conducted from a distance of 150 mm to standardised settings (Macro; Mid-SD resolution; 360° scans). Each cranial scan was split into 8 - 10 divisions. Light conditions were standardised and all scanning was done by the same person (BG), using the same scanner. Initially, two crania were repeatedly scanned in order to assess scanner accuracy. When digitizing specimens, a series of three sets of scans was recorded each time. Each of these series of scans was recorded along a different cranial axis, allowing for the entire specimen to be scanned (Fig 3.1). In combining these three scans each time to generate

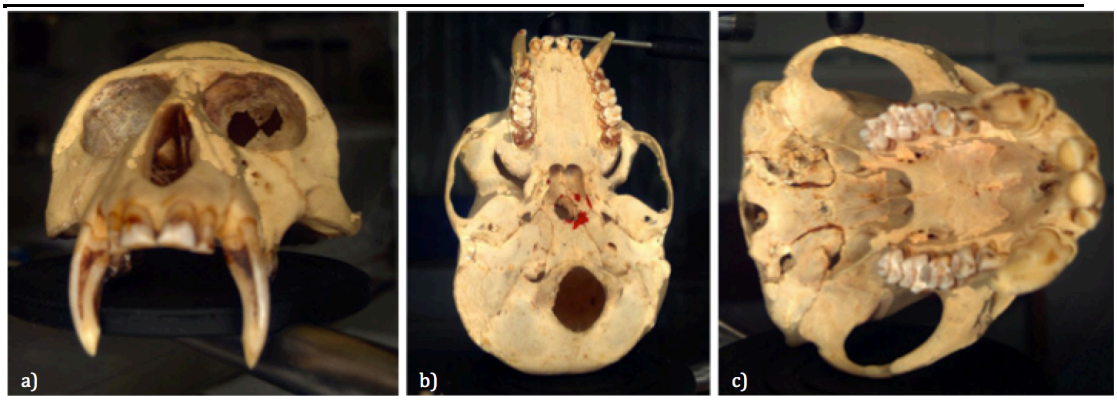

Figure 3.1. Scanner photos showing positioning of cranium required for one full cranial scan. Each of the three series of component scans (a-c) combines to permit every part of the cranium to be scanned. In each set, crania are secured using the turntable base and the armature attachment.

a complete scan, three arbitrary landmarks were manually plotted at corresponding places across the three component scan series. These scan alignment landmarks were positioned at clearly marked sutural junctions (Type 1 landmarks) or on scanned written labels, which were then aligned, trimming off unwanted scanned geometry accrued during the

component scans. To assess repeatability of scanning, the mean landmark standard deviation (mm) was calculated for two adult male Caribbean *Chlorocebus* crania (Table 3.2). Each was scanned 10 times and ascribed the same three landmarks each time. To further ensure a reliably high level of scanning repeatability, scans were only included that were aligned to an accuracy of 0.005 inches (0.13 mm) or higher (K. Balolia 2017, pers.comm. 1 March).

Table 3.2. Repeatability of scanning as shown through the mean landmark standard deviation (mm) for the three referenced landmarks digitized on two adult male *Chlorocebus* crania, each a total of ten times. Average error (SD) is calculated as a result across ten samples and all three referenced landmarks. ZTS: Zygo-Temp. Superior, RH: Rhinion.

Landmarks	ZTS (R)	ZTS (L)	RH	Ave. error
Specimen 1	0.239	0.151	0.274	0.221
Specimen 2	0.293	0.107	0.240	0.213

3.2.3 Landmarks

For each specimen, 69 cranial standard landmarks were recorded in the form of three-dimensional (3D) coordinate data (Frost *et al*. 2003; Cardini *et al*. 2007; Baab, 2008; Elton *et al*. 2010; Gilbert, 2010). The landmarks used are shown in Figure 3.2 and Table 3.3. Thirty two of the landmarks were collected from the dorsal aspect of the cranium, when viewed in either the Frankfurt Horizontal or from an anterior view and 37 landmarks were collected from a ventral aspect of the cranium while it was mounted approximately in *norma basilis*. All landmarks were ascribed by the same

person (BG), using Landmark (IDAV 3.6) software. To estimate the overall precision for this study, two specimens were measured ten times each (Table 3.2) following von Cramon-Taubadel *et al*. 2007. Overall precision across all coordinates was 0.213 mm.

The landmarks were chosen to cover all the main modular regions of the cranium (e.g. auditory region, facial zone, basicranium) in general respects. Landmarks were not located at points where ontogenetic effects such as wear due to varying diets were likely (e.g. on teeth or condylar processes). Landmarks were recorded on both the left and right side to record any structural asymmetry (Ashton and Zuckerman, 1951*b*; Ashton *et al*. 1979). Cranial landmarks were typically situated at sites where multiple sutures conjoined (Type 1) where possible or (Type 2) at points of maximum curvature.

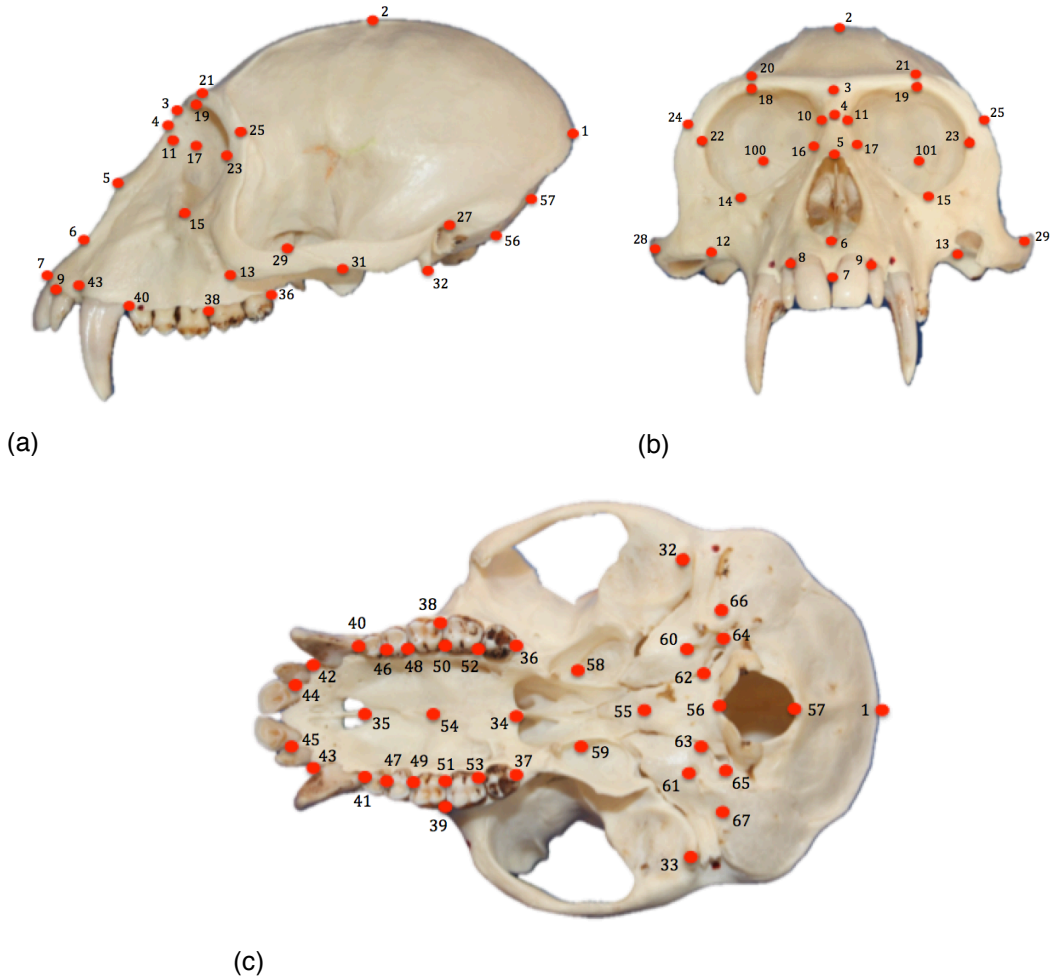

Figure 3.2. Cranial landmarks used in this study; (a) lateral view; (b) frontal view; (c) basicranial view. See also Table 3.3.

Table 3.3. Name, number and definition of cranial landmarks. The terms 'anterior' and 'posterior' are used with reference to Fig. 3.2.

No.	Landmark	Description
Midline positioning – cranial		
1	Inion (IN)	Most posterior point of cranium **(F)**
2	Bregma (BR)	Junction (ectocranial) of coronal and sagittal sutures
3	Glabella (GL)	Most anterior midline point on frontal bone (above frontonasal suture) **(F)**
4	Nasion (NA)	Fronto-nasal suture in midline, where 2 nasals and frontal intersect
5	Rhinion (RH)	Most anterior point in midline on nasals
6	Nasospinale (NS)	Inferior-most midline point of piriform aperture
7	Prosthion (PR)	Anteroinferior point on projection of premaxilla between central incisors
34	Incisivion (IV) *	Midline point at anterior-most point of the maxilla (posterior end of incisive foramen)
35	Staphylion (ST)	Midline point on palate n line tangent to anterior-most point on choanae
54	Maxipal (MXP)	Meeting of maxilla and palatine (in midline)
55	Basimidline (BML)	Meeting point between basisphenoid and basioccipital (in midline)
56	Basion (BA)	Anterior-most point of foramen magnum
57	Opisthion (OP)	Posterior-most point of foramen magnum

Table 3.3. *contd.*

Bilateral positioning – cranial

8-9	Prosthion 2 (PR2)	Antero-inferiormost point on premaxilla, between central & lateral incisors
10-11	Premax-max superior (PMS)	Where premaxillo-maxillary suture meets nasal bone (or aperture)
12-13	Zygo-max inferior (ZMI)	Anterinferior point of zygomaticomaxillary suture, in antero-lateral view
14-15	Zygo-max superior (ZMU)	Anterosuperior point of zygomaticomaxillary suture (taken at orbit rim)
16-17	Dacryon (DAC)	Frontal, lacrimal & maxilla junction (where lacrimomaxillary suture meets frontal)
18-19	Mid-torus inferior (MTI)	Inferior margin point of supraorbital torus (sup. margin of orbit) approx. orbit centre
20-21	Mid-torus superior (MTS)	Superior to MTI on superior most point of supraorbital torus **(F)**
22-23	Frontomalare orbitale (FMO)	Where frontozygomatic suture crosses the inner orbital rim
24-25	Frontomalare temporale (FMT)	Where frontozygomatic suture crosses the temporal line/lateral edge of zygoma
26-27	Porion (PO)	Top of auditory meatus **(F)**
28-29	Zygo-temp superior (ZTS)	Superior point of zygomatico-temporal suture on lateral face of zygomatic arch
30-31	Zygo-temp inferior (ZTI)	Inferolateral point of zygomaticotemporal suture on lateral face of zygomatic arch
32-33	Postglenoid (PG)	Tip (or midpoint) of area
36-37	Distal M3 (MD3)	Distal midpoint projected (laterally) onto alveolar margin
38-39	M1-2 contact (M12)	Projected (laterally) onto alveolar margin
40-41	Mesial P3 (MP3)	Most mesial point on P3 alveolar, projected onto alveolar margin
42-43	Premax-max inferior (PMI)	Where premaxillomaxillary suture crosses alveolar margin
44-45	Prosthion2 (PR2)	Antero-inferior point on projection of pre-maxilla between central incisors
46-53	Alveolar contacts (AVC)	Contact points between adjacent pre-molars/molars (lingual projection on alveolar margin)
58-59	Medialtemp (MTP)	Most medial point of the petrous part of the temporal bone.
60-61	Carotid foramen (CF)	Anterior side of carotid foramen
62-63	Medialjug (MJG)	Medial extremities of jugular foramen
64-65	Distaljug (DJG)	Distal extremities of jugular foramen
66-67	Stylomastoid foramen (STF)	Stylomastoid foramen
100-101	Optic foramen (OF)	Superior point of optic foramen

* May need to extrapolate if broken/asymmetrical

(F) represents landmark positioning as viewed in the Frankfurt horizontal

In order to report on the repeatability of landmarks in the context of relevant sample variation, seven specimens (all adult males from St. Kitts) were each attributed landmarks and two repeats were made for each specimen (n = 3 for each individual). For each individual (and subsequent repeats), all landmarks were ascribed, to test repeatability in the context of overall variation. This was done through the use of a Principal

Components Analysis (Fig 3.3) and a subsequent visual assessment on the graphical output of the PCA. Overall, there appears to be a high fidelity in terms of the repeatability of landmarks. Only one (landmark 67) showed some level of variation. This landmark was based at the stylomastoid foramen. There is no clear reason why this particular (Type 2) landmark should show more variation than others.

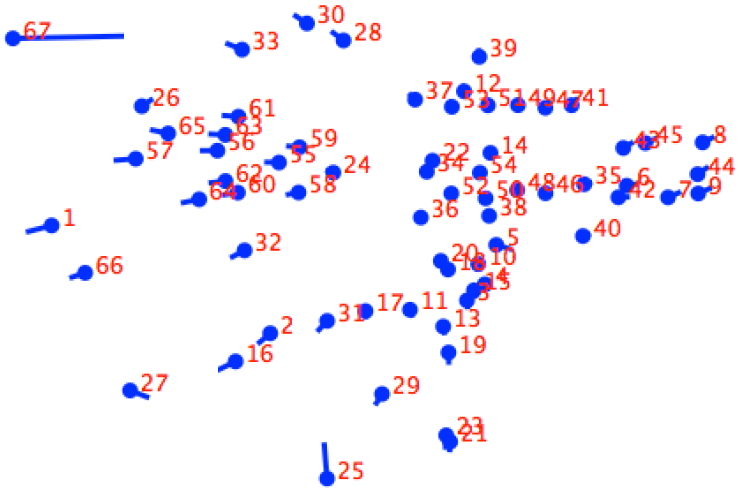

Figure 3.3. PCA results showing repeatability of landmarks for seven selected male Caribbean (St. Kitts) crania. The 'lollipop' vectors at each landmark show the variation for each position.

3.2.4 Data analyses

All data were analysed using 3D geometric morphometric techniques (Bookstein, 1994; Frost et al. 2003; Gilbert, 2010). 3D geometric morphometrics quantifies the 3D morphological structure of an organism, presenting and explaining the results of these analyses (Bookstein, 1989;

Adams *et al*. 2004; Cardini and Elton, 2008a; Gilbert, 2010). The landmark data were imported into the software package MORPHOLOGIKA 2.5 (Higgins and Jones, 1998; 2006), and Generalised Procrustes Analysis (GPA) were performed on 1) all male samples, 2) all female samples, 3) African male samples and 4) both sexes (combining African and Caribbean) pooled simultaneously, permitting four sets of analyses (Frieβ and Baylac, 2003; Slice, 2006; Badawi-Fayad and Cabanis, 2007). Male and female samples were separated to avoid the possibility of extraneous results from the high degree of sexual dimorphism that is characteristically seen in cercopithecine crania (Cardini and Elton, 2008). GPA works by reducing all landmark configurations to the same unit size, which removes any variation between specimens solely due to isometric size differences, through the use of centroid size (Rohlf and Slice, 1990; Cardini *et al*. 2007), where centroid size is the square root of the summed squared Euclidean distances from each landmark to the centroid of the landmark configuration. After adjustments for size, GPA uses a least squares criterion that minimises any remaining residual differences between configurations due to translation and rotation (Frost *et al*. 2003). Any remaining variation between landmark positions (i.e. the Procrustes residuals) can then be attributed to shape alone.

Following GPA (Fig 3.4), MORPHOLOGIKA was used to project the Procrustes residuals into a co-linear shape space, which were then subjected to a principal components analysis (PCA), with a table of

principal components (PC) scores produced for each specimen per analysis: male crania, female crania, all crania (from African and the Caribbean) and African male crania. PCA finds hypothetical variables (PCs) accounting for as much as possible of the variance in multivariate data (Davis, 1986; Harper, 1999). The use of PCA enables the major aspects of shape variation between specimens to be examined in a hierarchical fashion and determine whether the shape of one set of specimens is comparable with the shape of another set. The first PC describes the primary axis of shape variation between specimens (size already having been controlled for via GPA). The second PC describes the second most dominant aspects of variation, with subsequent PCs explaining sequentially smaller aspects of the overall variation. PCAs were based on the residuals from regressions of Procrustes coordinates on centroid size, with a summary table being produced for each specimen per analysis: all samples, all males, all females, and African males. Multivariate regression analysis were used to assess the significance of shape differences across the samples and between the ascribed groups: all samples (PCs 1 – 87); all male samples (PCs 1 - 46); female Caribbean samples (PCs 1 - 16); and male African samples (PCs 1 - 21). Eigenvalues and the loadings from the Eigenvectors for each PC (for each grouping) were also included, representing the maximum variance or correlation uniquely associated with the sample and the separate component roots, respectively. The variance and cumulative variance for each PC for each group was also included. In order to assess for any

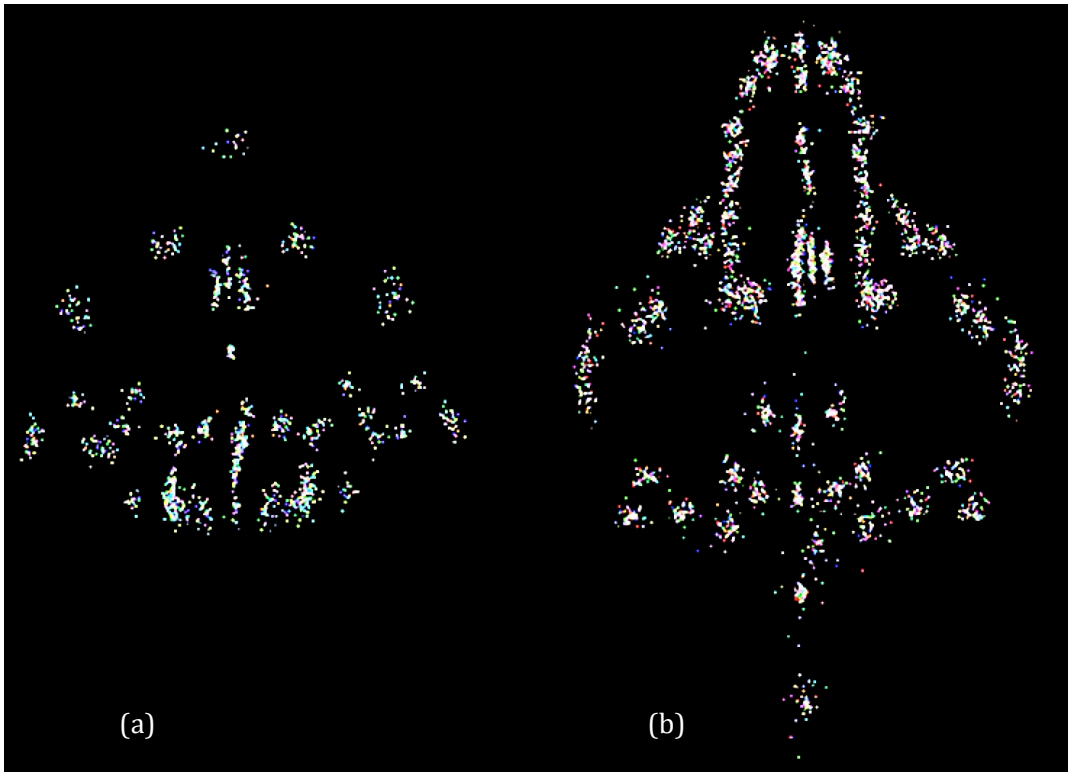

Figure 3.4. After Generalised Procrustes Analysis (GPA), showing; (a) frontal and (b) basicranial views. Taken from all combined female cranial data.

hierarchical groupings in the (multivariate) data sets created, hierarchical clustering analysis was used to produce a dendrogram, showing how data points (rows) are clustered. Using the software PAST 3.12, analysis was conducted using a Euclidean similarity index through the Ward's method. Bootstrapping resampling of columns was performed 5000 times. Within the data, columns were colour-divided to clearly show whether any differences between African and Caribbean crania existed. One-way ANOVA tests using SPSS 13.0 were used to assess differences between Caribbean and African and male and female crania.

Fluctuating (or bilateral) asymmetry in biological structures such as invertebrate wings and carapaces and vertebrate crania has been routinely used as a measure of developmental instability (Van Valen, 1962; Palmer and Strobeck, 1986; Graham et *al*. 2010), which can be correlated to various environmental or genetic factors. Such asymmetry is used to gauge developmental instability on the idea that corresponding structures on the left and right side (of a median line) are independent copies of a structure that develop under the control of the same genome and under the same environmental conditions (Klingenberg and McIntyre, 1998).

Corresponding structures on different sides of a body would develop as identical copies of each other, if development were a completely deterministic process (Mardia et al. 2000). However, because development is not entirely deterministic, randomly-occurring fluctuations in these processes causes often minor deviations from such an idealised symmetrical structure. Because the two halves/parts of a structure (on the left and right side) develop separately from each other, these random fluctuations of developmental processes affect each copy separately and are thus likely to produce deviations from the 'perfect' (or symmetrical) phenotype. As a result, even the two halves of a single cranium may show small phenotypic variations between them, arising as quantifiable asymmetry. It is this type of asymmetry that can be used as an indicator of

developmental instability (Marchand et *al*. 2003; Sánchez-Chardi et *al*. 2013; Maestri et *al*. 2015).

To analyse fluctuating asymmetry, a two-factor, mixed-effect ANOVA with individuals and sides as the two factors is used (Leamy, 1984; Palmer and Strobeck, 1986). The main effect of individuals results from variation in their left–right averages of trait values. The main effect of side reflects the average difference between left and right sides, and therefore represents directional asymmetry. The individual-by-side interaction is due to differences among individuals in their left–right asymmetries, and therefore stands for fluctuating asymmetry. This Procrustes ANOVA method can be incorporated into studies of fluctuating asymmetry when using geometric morphometrics (Klingenberg and McIntyre, 1998; Klingenberg et *al*. 2002). Because the calculations underlying the computations of the Procrustes superimposition are based on sums of squared deviations, it is compatible with the sums of squares used in conventional ANOVA. One of the key points of this type of analysis is that in the calculation of Procrustes distances, squared coordinate differences are added up across all coordinates of all the landmarks (Owen et *al*. 2014; Klingenberg, 2015). Calculating Procrustes sums of squares and using them to compute Goodall's *F* values allows for statistical tests of the effects in a Procrustes ANOVA that directly extends Goodall's use of ANOVA in the context of asymmetry analyses (Goodall, 1991; Klingenberg et *al*. 2002). Mean

squares are obtained by dividing the Procrustes sums of squares by the degrees of freedom for the respective effects.

3.3 Results

In incorporating the pooled data from all males and females from the three African species and the three Caribbean populations (Table 3.4), the first 35 (out of 87) PCs explained over 95% of the variance among cranial shape (PC1 λ= 0.0063, PC2 λ= 0.00089). Plotting the first three PCs (Fig 3.5.1 and Fig 3.5.2), which represent 57.81% of the cumulative variance shows that there is a marked difference in terms of cranial shape in some of the Caribbean population when comparing them to African species. Despite an apparent overlap between the shape of crania originating from Nevis and those from African *C. sabaeus*, two of the three Caribbean populations are distinct. Crania from both the Barbados and St Kitts populations are separated from the others but are in close association with those from Nevis and African *C. sabaeus*. Data from African *C. tantalus* and *C. cynosuros* are largely distinct from the other samples, although there is a little overlap from some of these samples. Some crania (e.g. *C. cynosuros*) typically have narrower zygomatic arches and are more vaulted, whereas others (e.g. Barbados) are characterised as having very broad zygomatic arches and crania that are less domed. The crania from St Kitts have faces that are noticeably broader than those from elsewhere in the Caribbean and Africa.

Table 3.4. Table summarising results of PCA for cranial shape for all samples: males, females, African samples and Caribbean samples. Results from this PCA are based on Procrustes residuals following full GPA (i.e. including scaling, translation and rotation . The table shows principal components (PC), eigenvalues, the percentage of variance attributed to each PC and the cumulative percentage of variance.

PC	Eigenvectors			Eigenvalue	% Variance	Cum. % variance
	X_1	X_2	X_3			
PC 1	0.010	0.035	0.126	0.0063	45.39	45.39
PC 2	0.067	-0.024	0.102	0.0008	6.42	51.81
PC 3	0.039	-0.020	0.068	0.0008	6.00	57.81
PC 4	0.041	0.038	-0.098	0.0006	4.61	62.43
PC 5	-0.016	-0.010	-0.020	0.0005	3.47	65.90
PC 6	-0.048	-0.040	-0.055	0.0004	3.03	68.93
PC 7	-0.045	-0.017	-0.021	0.0003	2.45	71.39
PC 8	-0.041	-0.024	-0.040	0.0003	2.23	73.62
PC 9	0.040	-0.011	0.080	0.0002	2.13	75.75
PC 10	0.033	-0.016	0.057	0.0002	1.92	77.67
PC 11	0.032	0.005	-0.040	0.0002	1.61	79.29
PC 12	0.023	-0.028	0.027	0.0002	1.49	80.78
PC 13	0.049	0.019	0.015	0.0001	1.33	82.11
PC 14	0.035	0.008	0.011	0.0001	1.11	83.23
PC 15	0.024	-0.021	0.062	0.0001	1.06	84.29
PC 16	0.021	-0.026	0.051	0.0001	1.00	85.30
PC 17	0.004	0.027	0.140	0.0001	0.90	86.20
PC 18	0.007	0.014	0.117	0.0001	0.82	87.03
PC 19	0.008	0.017	0.160	0.0001	0.78	87.81
PC 20	0.009	0.002	0.142	0.0001	0.70	88.52
PC 21	0.012	0.023	0.044	0.0001	0.64	89.17
PC 22	0.020	-0.005	0.013	0.0001	0.61	89.79
PC 23	0.019	0.053	0.017	0.0001	0.56	90.35
PC 24	0.020	0.007	-0.009	0.0001	0.55	90.91
PC 25	0.001	0.022	-0.075	0.0001	0.50	91.41
PC 26	0.030	0.361	-0.038	0.0001	0.45	91.87
PC 27	0.037	-0.002	-0.088	0.0001	0.43	92.31
PC 28	-0.008	0.030	0.089	0.0001	0.40	92.71
PC 29	0.014	0.028	-0.016	0.0001	0.39	93.11
PC 30	-0.021	0.047	0.119	0.0001	0.37	93.48
PC 31	0.000	-0.018	-0.067	0.0001	0.34	93.83
PC 32	-0.029	0.039	-0.119	0.0001	0.32	94.15
PC 33	-0.027	0.017	-0.146	0.0001	0.31	94.46
PC 34	-0.004	0.025	-0.137	0.0001	0.29	94.75
PC 35	-0.069	-0.007	-0.055	0.0001	0.27	95.03
PC 36	-0.076	0.021	-0.045	0.0001	0.26	95.30
PC 37	-0.057	-0.002	-0.082	0.0001	0.25	95.55
PC 38	-0.070	0.028	-0.072	0.0001	0.23	95.78
PC 39	-0.049	-0.021	-0.078	0.0001	0.22	96.00
PC 40	-0.055	0.018	-0.061	0.0001	0.21	96.22
PC 41	-0.048	-0.045	-0.083	0.0001	0.20	96.43
PC 42	-0.048	-0.014	-0.056	0.0001	0.20	96.63
PC 43	-0.065	-0.040	-0.047	0.0001	0.18	96.82

Table 3.4 *continued*

PC 44	-0.067	-0.031	-0.046	0.0001	0.17	96.99
PC 45	-0.051	0.004	-0.061	0.0001	0.17	97.16
PC 46	-0.045	0.019	-0.152	0.0001	0.15	97.32
PC 47	-0.048	-0.009	-0.072	0.0001	0.15	97.47
PC 48	-0.050	0.134	-0.139	0.0001	0.14	97.62
PC 49	-0.054	-0.008	-0.063	0.0001	0.13	97.75
PC 50	-0.057	-0.016	-0.135	0.0001	0.13	97.88
PC 51	-0.051	-0.003	-0.060	0.0001	0.12	98.01
PC 52	-0.072	0.011	-0.071	0.0001	0.11	98.12
PC 53	0.003	-0.008	-0.044	0.0001	0.11	98.23
PC 54	0.028	-0.015	-0.076	0.0001	0.10	98.34
PC 55	0.025	-0.035	-0.092	0.0001	0.10	98.44
PC 56	-0.012	-0.011	-0.020	0.0001	0.10	98.53
PC 57	0.038	0.003	-0.090	0.0001	0.10	98.63
PC 58	0.031	0.014	-0.089	0.0001	0.09	98.72
PC 59	0.036	-0.036	-0.035	0.0001	0.09	98.81
PC 60	0.040	-0.026	-0.019	0.0001	0.08	98.89
PC 61	0.024	-0.009	-0.028	0.0001	0.08	98.97
PC 62	0.023	-0.027	0.027	0.0001	0.08	99.04
PC 63	0.013	0.004	-0.061	0.0001	0.07	99.11
PC 64	0.033	0.003	0.150	0.0001	0.06	99.18
PC 65	0.016	-0.058	-0.084	0.0001	0.06	99.24
PC 66	0.018	-0.016	-0.064	0.0001	0.05	99.30
PC 67	0.031	0.013	-0.025	0.0001	0.05	99.36
PC 68	0.009	-0.052	0.001	0.0001	0.05	99.41
PC 69	0.004	-0.033	-0.005	0.0001	0.05	99.47
PC 70	0.009	-0.024	-0.027	0.0001	0.05	99.52
PC 71	-0.005	-0.033	-0.030	0.0001	0.04	99.56
PC 72	-0.007	-0.027	-0.032	0.0001	0.04	99.61
PC 73	0.002	-0.021	0.002	0.0001	0.04	99.65
PC 74	-0.026	-0.013	-0.036	0.0001	0.04	99.69
PC 75	0.006	-0.005	0.028	0.0001	0.04	99.72
PC 76	0.006	-0.012	0.006	0.0001	0.03	99.76
PC 77	0.008	-0.014	0.055	0.0001	0.03	99.79
PC 78	-0.003	0.007	-0.029	0.0001	0.02	99.82
PC 79	-0.001	-0.004	0.029	0.0001	0.02	99.85
PC 80	-0.010	0.042	-0.057	0.0001	0.02	99.87
PC 81	0.002	-0.006	0.035	0.0001	0.01	99.89
PC 82	0.009	-0.014	-0.003	0.0001	0.01	99.92
PC 83	0.013	-0.010	0.035	0.0001	0.01	99.94
PC 84	-0.025	0.015	0.031	0.0001	0.01	99.96
PC 85	0.012	-0.012	0.033	0.0001	0.00.	99.97
PC 86	-0.020	0.014	0.044	0.0001	0.00.	99.98
PC 87	0.027	-0.019	0.045	0.0001	0.00.	100.00

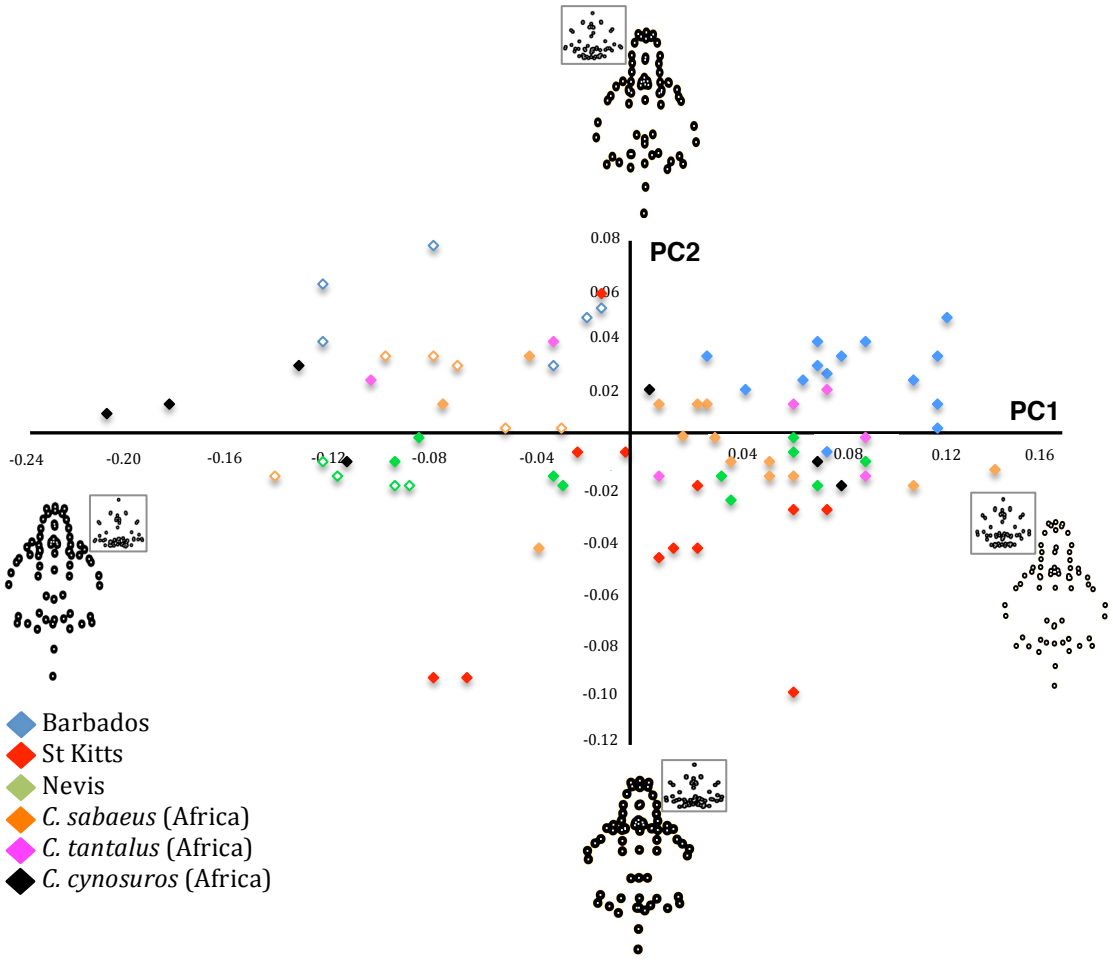

Figure 3.5.1. Plot of PC 1 vs. PC 2 for cranial shape from all samples: males, females, African samples and Caribbean samples, incorporating size and shape after GPA. Data from male samples are grouped in coloured boundaries to show approximate groupings. Male samples are represented by shaded boxes and females represented by unshaded boxes. Point-based images illustrating the changes in basicranial and maxilla-facial (inset) shape across each axis are indicated at the ends of each axis. Point image scales: ventral view 1 : 5, dorsal view 1 : 7.

In incorporating the data from all males from the three African species and the three Caribbean populations (Table 3.5), the first 29 (out of 46) PCs explained >95% of the variance among cranial shape (PC1 λ= 0.0015, PC2 λ= 0.0012). Plots of the first three PCs (Fig 3.6.1 and Fig 3.6.2), which represent 40.08% of the cumulative variance shows that there is a general pattern of overlap between many of the samples from the various sample populations. Some crania (e.g. *C. cynosuros*) typically have narrower zygomatic arches and are more vaulted, whereas others (e.g. the

Barbados population) are characterised as having very broad zygomatic arches and crania that are less domed. African *C. cynosuros* samples do largely sit separately from the others but these are still interspersed with

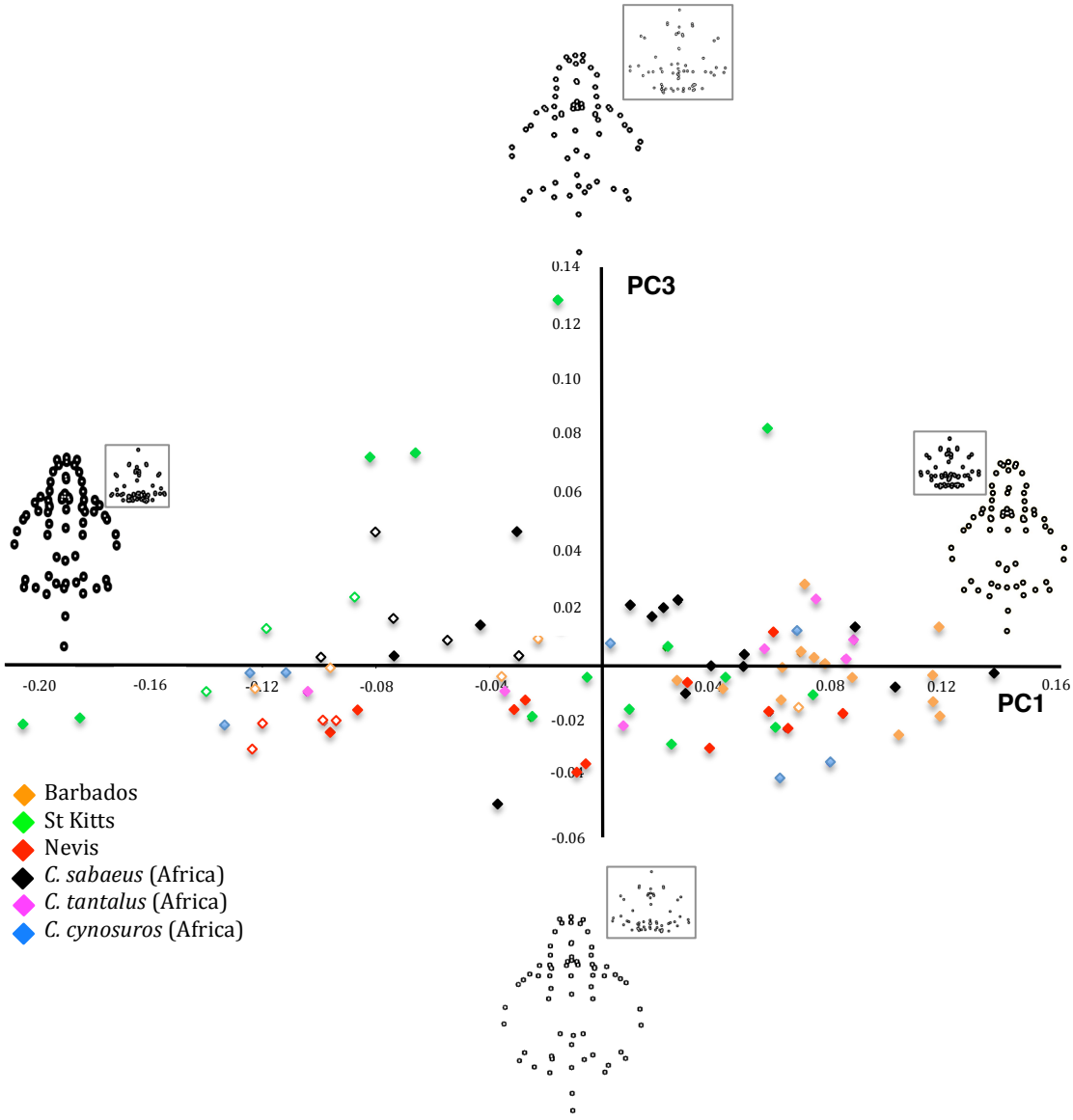

Figure 3.5.2. Plot of PC 1 vs. PC 3 for cranial shape from all samples: males, females, African samples and Caribbean samples, incorporating size and shape after GPA. Data from male samples are grouped in coloured boundaries to show approximate groupings. Male samples are represented by shaded boxes and females represented by unshaded boxes. Point-based images illustrating the changes in basicranial and maxilla-facial (inset) shape across each axis are indicated at the ends of each axis. Point image scales: ventral view 1 : 5, dorsal view 1 : 7.

other samples. Whilst there is overlap in the variance based on shape between the numerous populations, some Caribbean populations are distinct from one another. Those crania from Barbados are distinct from Nevis and St Kitts (and these samples additionally appear separate from one another). Samples from Barbados, Nevis, *C. sabaeus* and *C. tantalus* show little variance within their separate populations in terms of shape, whereas African *C. cynosuros* samples display a moderate amount of variation. With data points widely spread across the graph, crania from St Kitts show a large degree of variance. Although there appears to be some marked variation within the population, St Kitts monkeys are largely different in that they have a short muzzle and more domed crania, with broader zygomatic arches. Crania from Nevis are more prognathic, with a longer, narrower muzzle. They have narrower zygomatic arches and are less domed. Crania from Barbados have an even more pronounced muzzle and a very low-domed cranium. Whilst the crania from Nevis and Barbados are distinct from one another, they do share some similarities with crania from African species. Nevis crania tend to fall in with *C. tantalus*, whereas Barbados crania most closely resemble *C. sabaeus*.

Table 3.5. Table summarising results of PCA for male cranial shape from Caribbean (Nevis, St. Kitts and Barbados) samples and African (*C. sabaeus*, *C. tantalus* and *C. cynosuros*) samples. Results from this PCA are based on Procrustes residuals following full GPA (i.e. including scaling, translation and rotation). The table shows principal components (PC), eigenvalues, the percentage of variance attributed to each PC and the cumulative percentage of variance.

PC	Eigenvector			Eigenvalue	% Variance	Cum. % variance
	x_1	x_2	x_3			
PC 1	0.064	0.069	0.059	1.50E-03	1.87E-01	1.87E-01
PC 2	-0.096	-0.113	0.027	1.22E-03	1.52E-01	3.39E-01

Table 3.5 *continued*

PC 3	-0.063	-0.074	0.019	5.55E-04	6.91E-02	4.08E-01
PC 4	-0.072	-0.016	-0.034	4.81E-04	5.98E-02	4.68E-01
PC 5	-0.007	0.032	-0.072	4.66E-04	5.79E-02	5.26E-01
PC 6	0.026	0.012	-0.115	3.68E-04	4.58E-02	5.72E-01
PC 7	0.025	0.010	-0.112	3.08E-04	3.84E-02	6.10E-01
PC 8	0.023	-0.001	-0.124	2.84E-04	3.54E-02	6.46E-01
PC 9	-0.048	-0.087	0.072	2.56E-04	3.18E-02	6.77E-01
PC 10	-0.042	-0.078	0.055	2.25E-04	2.80E-02	7.05E-01
PC 11	-0.060	-0.027	-0.010	2.00E-04	2.49E-02	7.30E-01
PC 12	-0.052	-0.033	0.007	1.94E-04	2.41E-02	7.54E-01
PC 13	-0.045	0.002	0.038	1.77E-04	2.20E-02	7.76E-01
PC 14	-0.034	0.008	0.030	1.72E-04	2.14E-02	7.98E-01
PC 15	-0.034	-0.072	0.057	1.54E-04	1.92E-02	8.17E-01
PC 16	-0.035	-0.068	0.040	1.37E-04	1.70E-02	8.34E-01
PC 17	0.001	-0.060	0.079	1.14E-04	1.42E-02	8.48E-01
PC 18	0.007	-0.071	0.063	9.90E-05	1.23E-02	8.61E-01
PC 19	-0.001	-0.059	0.075	9.30E-05	1.16E-02	8.72E-01
PC 20	0.001	-0.084	0.061	8.48E-05	1.06E-02	8.83E-01
PC 21	-0.021	-0.015	0.081	8.24E-05	1.03E-02	8.93E-01
PC 22	-0.017	-0.013	0.049	7.69E-05	9.57E-03	9.03E-01
PC 23	-0.030	0.000	0.051	7.28E-05	9.06E-03	9.12E-01
PC 24	-0.032	0.019	0.039	6.21E-05	7.73E-03	9.19E-01
PC 25	-0.006	0.015	-0.019	6.05E-05	7.53E-03	9.27E-01
PC 26	-0.004	0.018	-0.007	5.59E-05	6.95E-03	9.34E-01
PC 27	-0.076	-0.044	-0.013	5.20E-05	6.47E-03	9.40E-01
PC 28	-0.051	-0.113	-0.010	4.78E-05	5.95E-03	9.46E-01
PC 29	-0.064	-0.125	-0.036	4.41E-05	5.49E-03	9.52E-01
PC 30	-0.046	-0.199	-0.048	3.95E-05	4.92E-03	9.57E-01
PC 31	0.029	0.051	0.019	3.78E-05	4.70E-03	9.61E-01
PC 32	0.021	0.046	0.005	3.75E-05	4.66E-03	9.66E-01
PC 33	0.032	0.092	-0.054	3.42E-05	4.25E-03	9.70E-01
PC 34	0.010	0.048	-0.058	3.04E-05	3.78E-03	9.74E-01
PC 35	0.074	0.066	-0.068	2.68E-05	3.34E-03	9.77E-01
PC 36	0.084	0.073	-0.074	2.56E-05	3.18E-03	9.81E-01
PC 37	0.043	0.050	-0.097	2.54E-05	3.16E-03	9.84E-01
PC 38	0.047	0.044	-0.110	2.23E-05	2.78E-03	9.87E-01
PC 39	0.042	0.063	-0.082	2.09E-05	2.60E-03	9.89E-01
PC 40	0.051	0.065	-0.126	1.87E-05	2.33E-03	9.91E-01
PC 41	0.039	0.065	-0.126	1.70E-05	2.11E-03	9.94E-01
PC 42	0.044	0.075	-0.099	1.50E-05	1.87E-03	9.95E-01
PC 43	0.066	0.069	-0.110	1.39E-05	1.73E-03	9.97E-01
PC 44	0.054	0.052	-0.108	1.16E-05	1.44E-03	9.99E-01
PC 45	0.016	0.007	-0.051	1.10E-05	1.37E-03	1.00E+00
PC 46	0.036	0.054	-0.155	7.00E-12	8.71E-10	1.00E+00

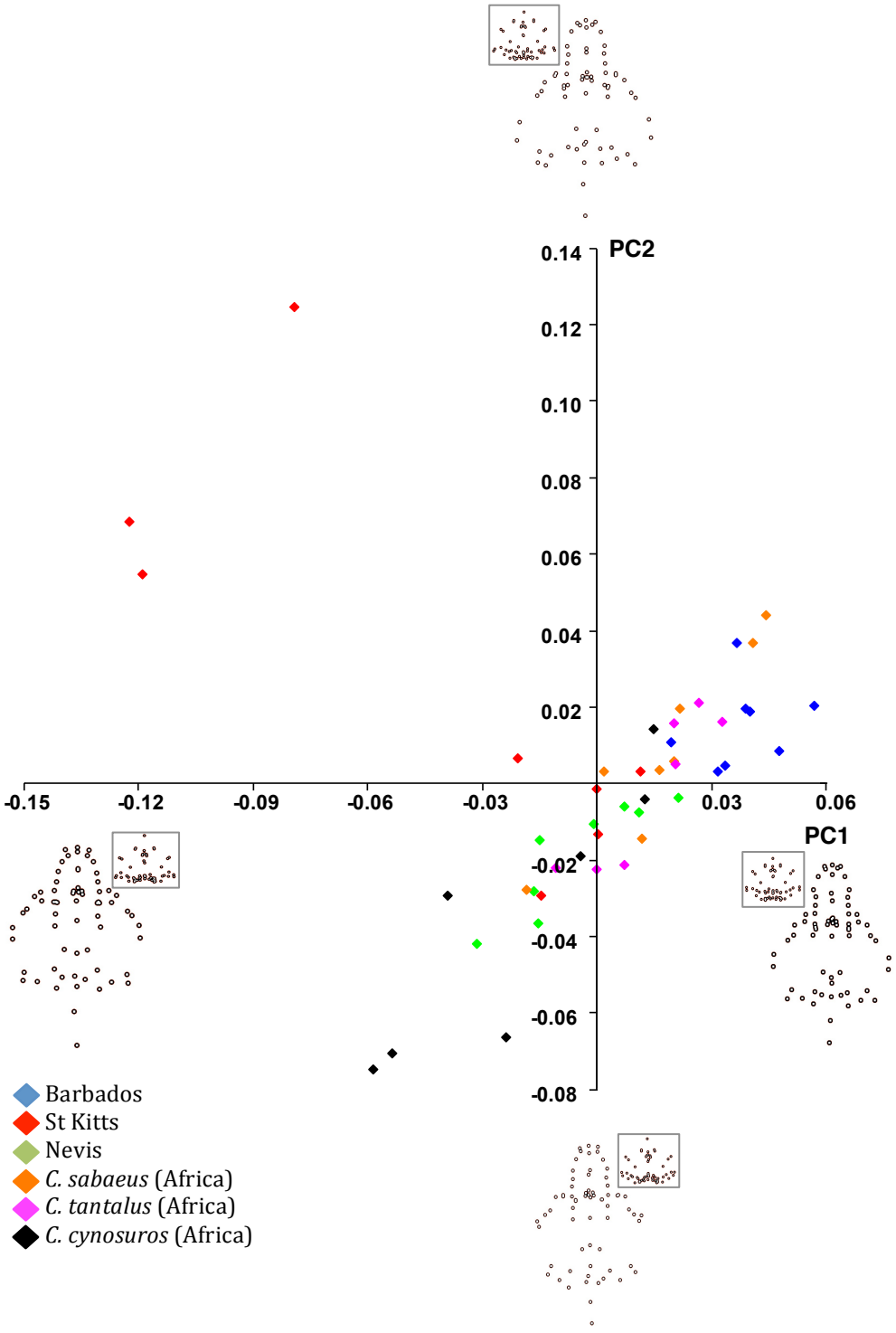

Figure 3.6.1. Plot of PC 1 vs. PC 2 for male cranial shape from Caribbean Nevis (green), St. Kitts (red) and Barbados (blue) samples and African *C. sabaeus* (orange), *C. tantalus* (pink) and *C. cynosuros* (black) samples, incorporating size and shape (after Procrustes superimposition). Male samples are represented by shaded boxes and females represented by unshaded boxes. Point-based images illustrating the changes in basicranial and maxilla-facial (inset) shape across each axis are indicated at the ends of each axis. Point image scales: ventral view 1 : 5, dorsal view 1 : 7.

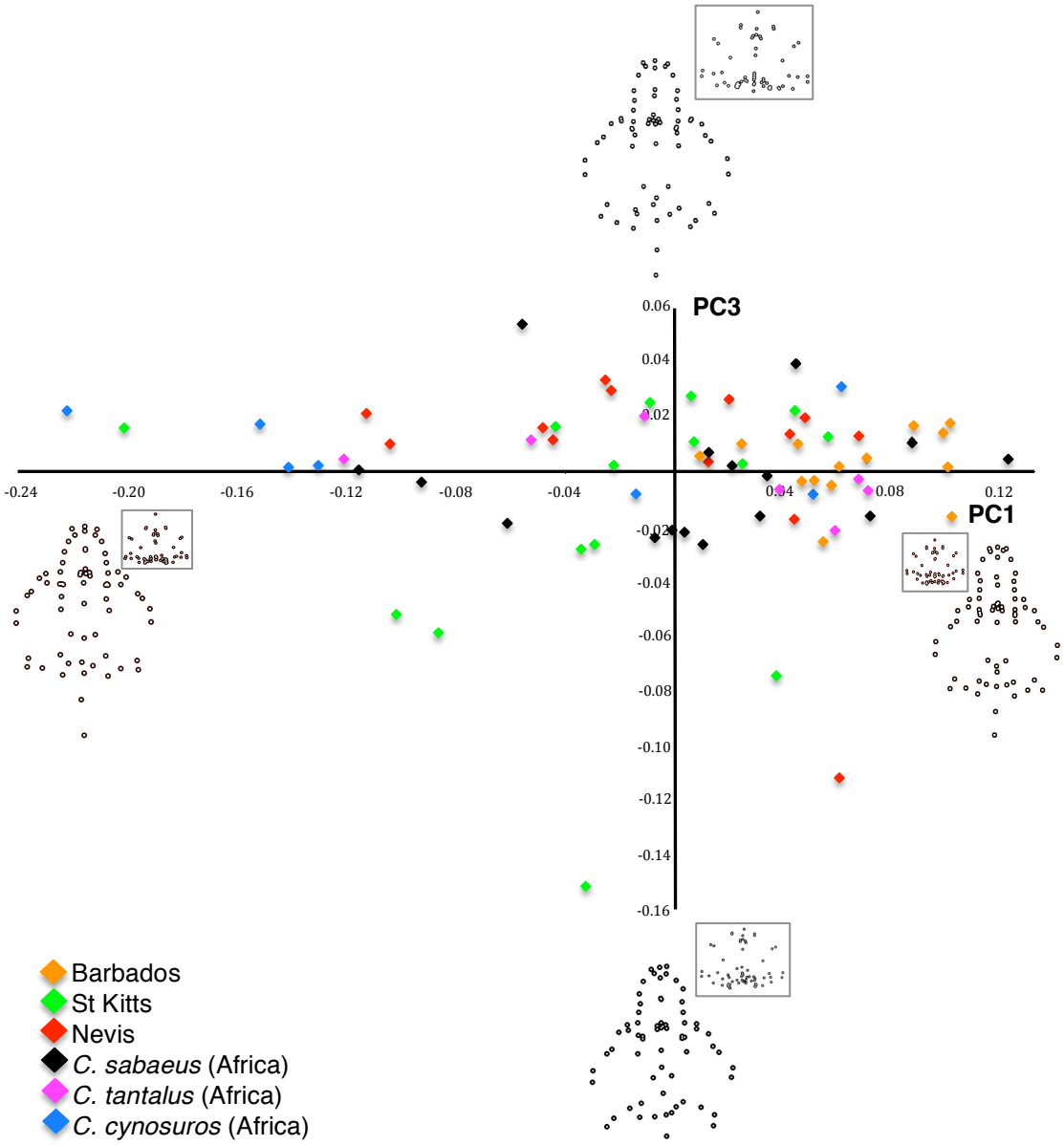

Figure 3.6.2. Plot of PC 1 vs. PC 3 for male cranial shape from Caribbean Nevis (green), St. Kitts (red) and Barbados (blue) samples and African *C. sabaeus* (orange), *C. tantalus* (pink) and *C. cynosuros* (black) samples, incorporating size and shape (after Procrustes superimposition). Male samples are represented by shaded boxes and females represented by unshaded boxes. Point-based images illustrating the changes in basicranial and maxilla-facial (inset) shape across each axis are indicated at the ends of each axis. Point image scales: ventral view 1 : 5, dorsal view 1 : 7.

In incorporating the data from all females from one African species (*C. sabaeus*) and the three Caribbean populations (Table 3.6), the first 12 (out of 16) PCs explained >95% of the variance among cranial shape (PC1 λ=

0.0022, PC2 λ= 0.00087). Plots of the first two PCs (Fig 3.7.1 and Fig 3.7.2), which represent 62.62% of the cumulative variance demonstrates that there is a clear pattern that female crania show marked differentiation between populations and species. Crania from Nevis and St Kitts appear close (in terms of shape) to one another and are largely separate from Barbados crania. Female crania from Nevis and St Kitts are similar to one another in that they are broader overall, with much wider zygomatic arches than the other crania. Additionally, they have a broader muzzle. In contrast to this, African *C. sabaeus* and Barbados crania appear to have narrower but much longer muzzles. Between these two populations, the Barbados crania have the longest, narrowest muzzles and are less vaulted than those from Nevis and St Kitts.

Table 3.6. Table summarising results of PCA for female cranial shape from Caribbean (Nevis, St. Kitts and Barbados) samples and African (*C. sabaeus*) samples. Results from this PCA are based on Procrustes residuals following full GPA (i.e. including scaling, translation and rotation). The table shows principal components (PC), eigenvalues, the percentage of variance attributed to each PC and the cumulative percentage of variance.

PC	Eigenvector			Eigenvalue	% Variance	Cum. % variance
	x_1	x_2	x_3			
PC 1	-0.021	-0.121	0.135	0.0022	37.61	37.61
PC 2	0.022	0.051	0.073	0.0009	15.12	52.74
PC 3	0.004	0.023	0.033	0.0006	9.87	62.62
PC 4	0.023	0.034	0.003	0.0004	7.08	69.70
PC 5	0.011	0.061	-0.076	0.0003	6.04	75.74
PC 6	0.054	0.048	-0.061	0.0003	4.65	80.40
PC 7	0.056	0.086	-0.117	0.0002	3.77	84.17
PC 8	0.038	0.045	-0.044	0.0002	3.20	87.38
PC 9	-0.041	-0.040	0.075	0.0002	2.75	90.13
PC 10	-0.047	-0.028	0.068	0.0001	2.08	92.21
PC 11	-0.031	-0.003	0.094	0.0001	1.81	94.02
PC 12	-0.026	0.037	0.068	0.0001	1.50	95.53
PC 13	0.016	0.041	0.056	0.0001	1.40	96.93
PC 14	-0.013	0.019	0.070	0.0001	1.21	98.15
PC 15	-0.051	-0.068	0.057	0.0001	0.96	99.12
PC 16	-0.050	-0.048	0.098	0.0001	0.87	99.99

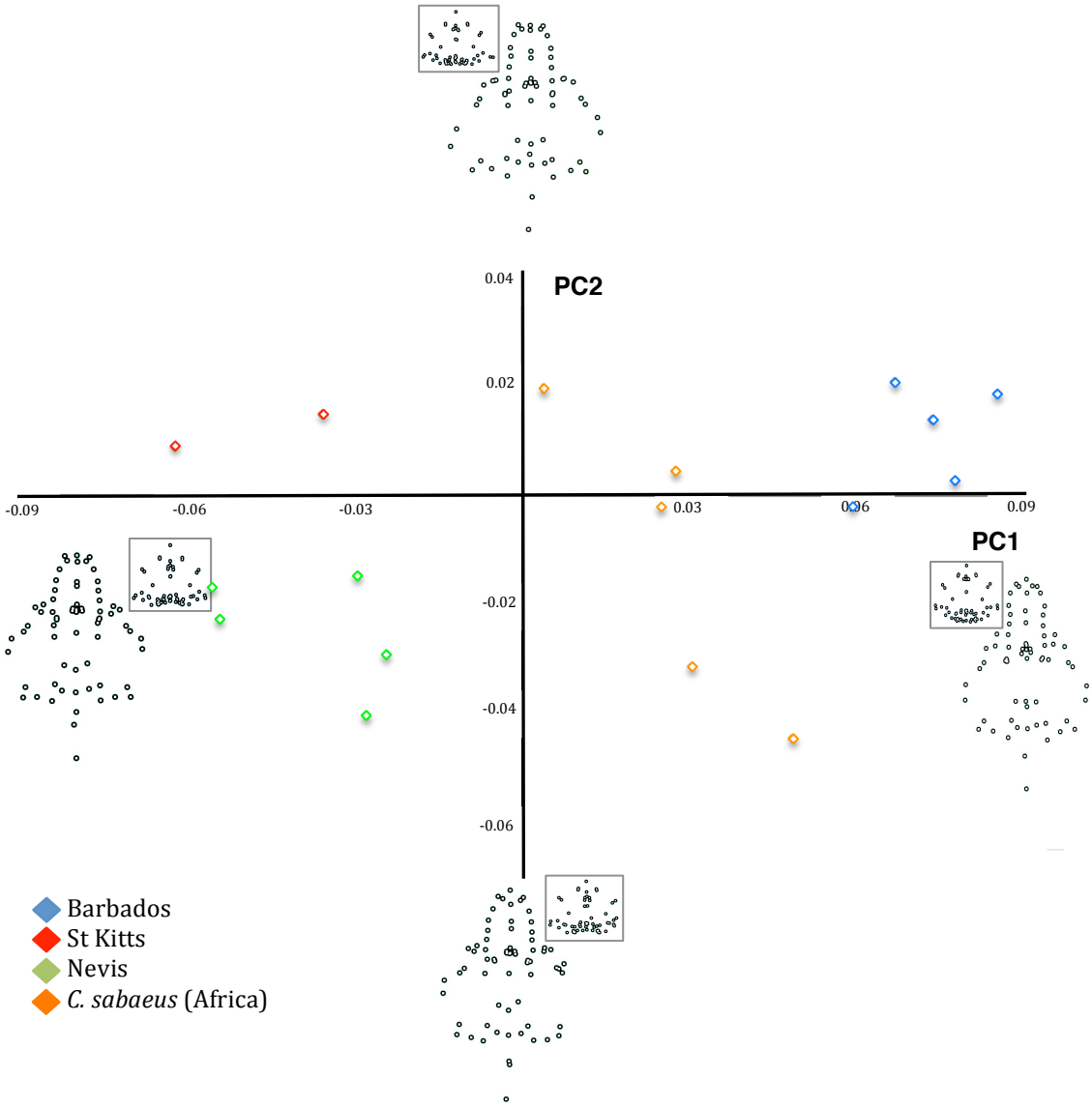

Figure 3.7.1. Plot of PC 1 vs. PC 2 for female cranial shape from Caribbean Nevis (green), St. Kitts (red) and Barbados (blue) samples and African *C. sabaeus* (orange) samples, incorporating size and shape (after Procrustes superimposition). Male samples are represented by shaded boxes and females represented by unshaded boxes. Point-based images illustrating the changes in basicranial and maxilla-facial (inset) shape across each axis are indicated at the ends of each axis. Point image scales: ventral view 1 : 5, dorsal view 1 : 7.

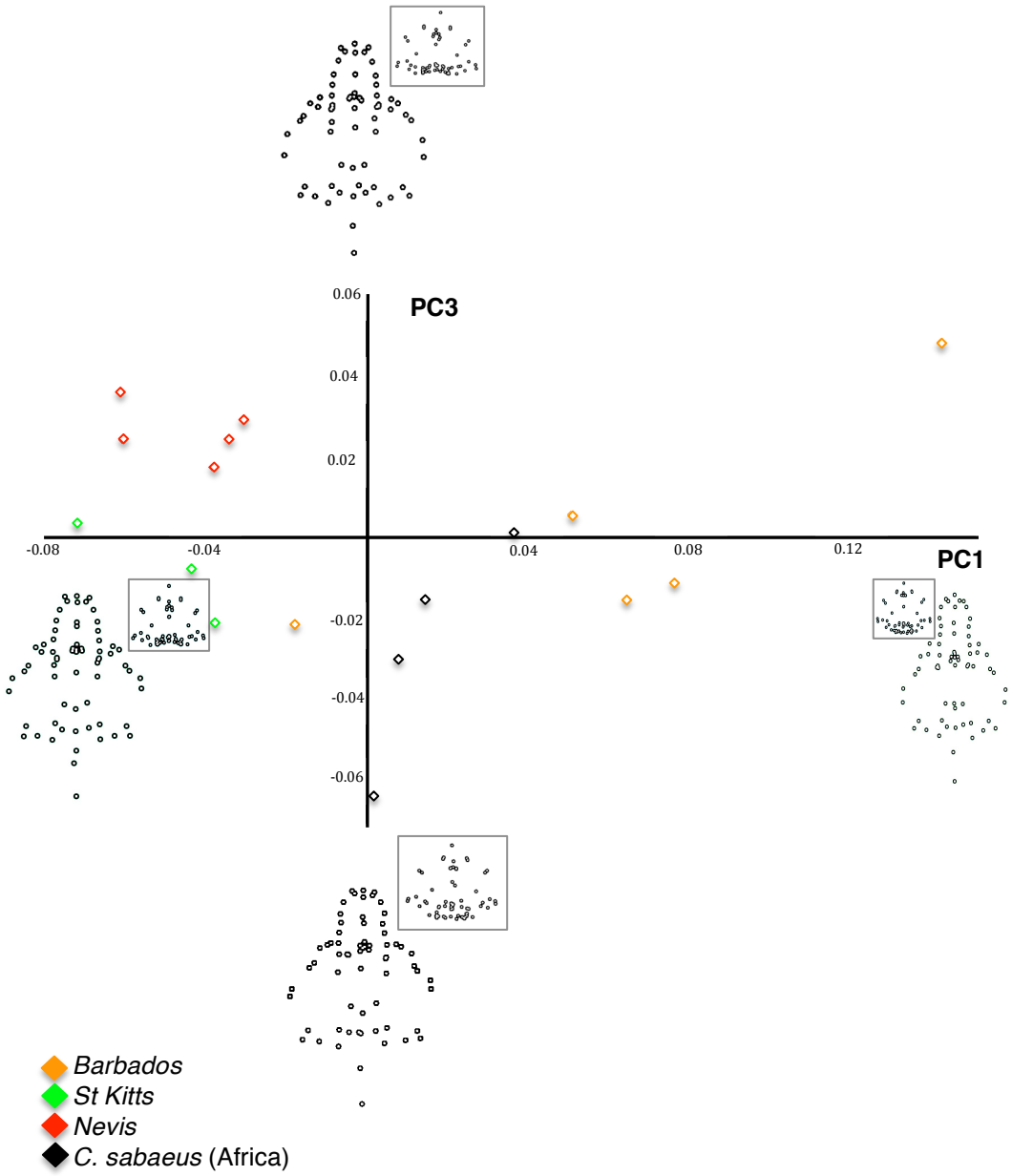

Figure 3.7.2. Plot of PC 1 vs. PC 2 for female cranial shape from Caribbean Nevis (green), St. Kitts (red) and Barbados (blue) samples and African *C. sabaeus* (orange) samples, incorporating size and shape (after Procrustes superimposition). Male samples are represented by shaded boxes and females represented by unshaded boxes. Point-based images illustrating the changes in basicranial and maxilla-facial (inset) shape across each axis are indicated at the ends of each axis. Point image scales: ventral view 1 : 5, dorsal view 1 : 7.

In incorporating the data from African males from the three African species *C. sabaeus*, *C. tantalus* and *C. cynosuros* (Table 3.7), the first 13 (out of

21) PCs explained >95% of the variance among cranial shape (PC1 λ= 0.010, PC2 λ= 0.0010). Plots of the first three PCs (Fig 3.8.1 and Fig 3.8.2), which represent 75.39% of the cumulative variance shows that there is a general pattern of overlap between many of the samples from these three species. African *C. cynosuros* samples do largely sit separately from the others but there is still some overlap with other species' samples. Samples from *C. sabaeus* and *C. tantalus* are hard to distinguish from one another, with both showing marked levels of variance in terms of shape. The crania from *C. cynosuros* appear to be more highly

Table 3.7. Table summarising results of PCA for African male cranial shape from African-sourced *C. sabaeus*, *C. tantalus* and *C. cynosuros* samples. Results from this PCA are based on Procrustes residuals following full GPA (i.e. including scaling, translation and rotation). The table shows principal components (PC), eigenvalues, the percentage of variance attributed to each PC and the cumulative percentage of variance.

PC	Eigenvector			Eigenvalue	% Variance	Cum. % variance
	x_1	x_2	x_3			
PC 1	0.010	0.035	0.126	0.0101	63.58	63.58
PC 2	0.067	-0.024	0.102	0.0010	6.39	69.97
PC 3	0.039	-0.020	0.068	0.0008	5.41	75.39
PC 4	0.041	0.038	-0.098	0.0005	3.50	78.89
PC 5	-0.016	-0.010	-0.020	0.0004	3.00	81.90
PC 6	-0.048	-0.040	-0.055	0.0004	2.87	84.77
PC 7	-0.045	-0.017	-0.021	0.0004	2.51	87.29
PC 8	-0.041	-0.024	-0.040	0.0003	2.09	89.39
PC 9	0.040	-0.011	0.080	0.0002	1.79	91.19
PC 10	0.033	-0.016	0.057	0.0002	1.36	92.56
PC 11	0.032	0.005	-0.040	0.0001	1.22	93.78
PC 12	0.023	-0.028	0.027	0.0001	1.13	94.91
PC 13	0.049	0.019	0.015	0.0001	0.93	95.85
PC 14	0.035	0.008	0.011	0.0001	0.86	96.71
PC 15	0.024	-0.021	0.062	0.0001	0.76	97.48
PC 16	0.021	-0.026	0.051	0.0001	0.66	98.14
PC 17	0.004	0.027	0.140	0.0001	0.51	98.66
PC 18	0.007	0.014	0.117	0.0001	0.39	99.05
PC 19	0.008	0.017	0.160	0.0001	0.38	99.44
PC 20	0.009	0.002	0.142	0.0001	0.31	99.75
PC 21	0.012	0.023	0.044	0.00001	0.24	100

vaulted than those from *C. sabaeus* and *C. tantalus* and have a thinner facial region. Crania from *C. sabaeus* and *C. tantalus* appear to have wider zygomatic arches also. The main difference between *C. sabaeus* and *C. tantalus* is that *C. sabaeus* crania have a wider and shorter muzzle.

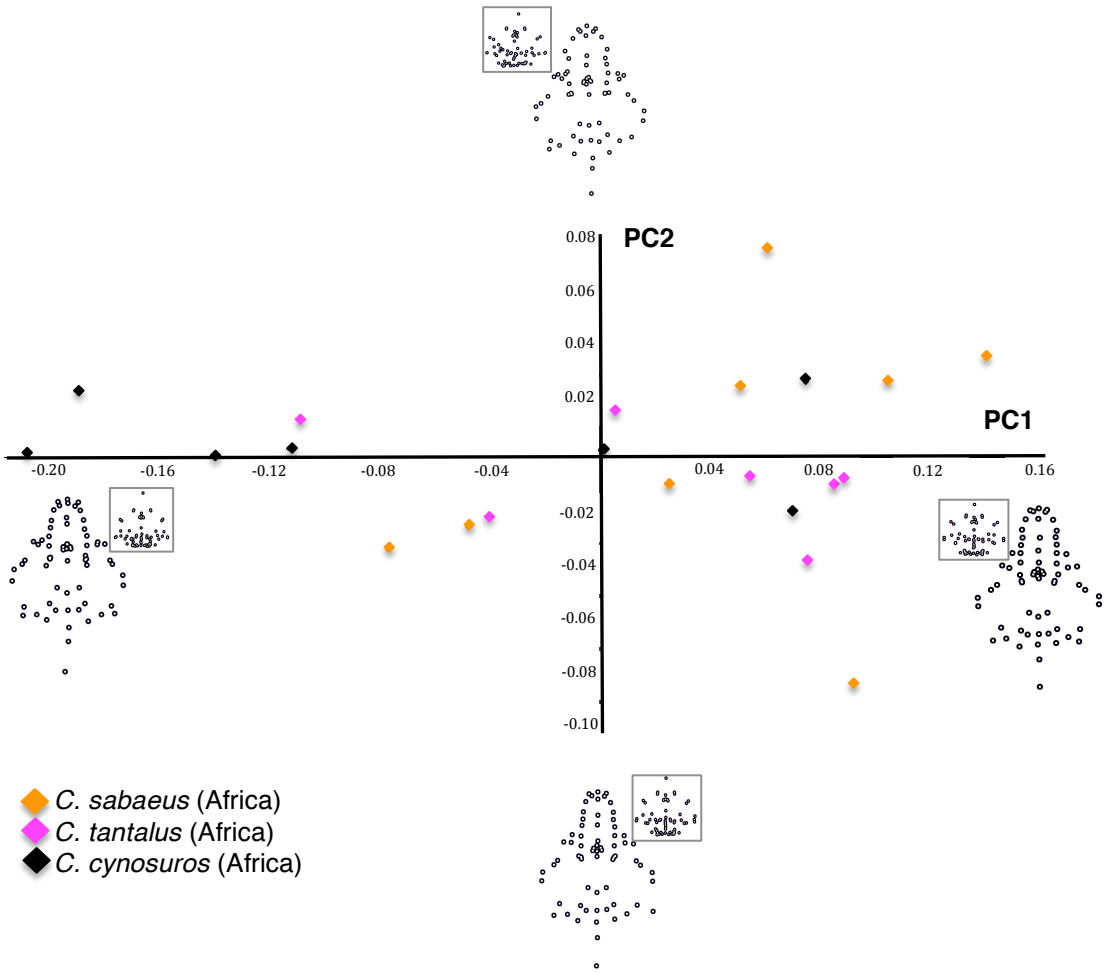

Figure 3.8.1. Plot of PC 1 vs. PC 2 for African male cranial shape from African-sourced *C. sabaeus* (orange), *C. tantalus* (pink) and *C. cynosuros* (black) samples, incorporating size and shape (after Procrustes superimposition). Male samples are represented by shaded boxes and females represented by unshaded boxes. Point-based images illustrating the changes in basicranial and maxilla-facial (inset) shape across each axis are indicated at the ends of each axis. Point image scales: ventral view 1 : 5, dorsal view 1 : 7.

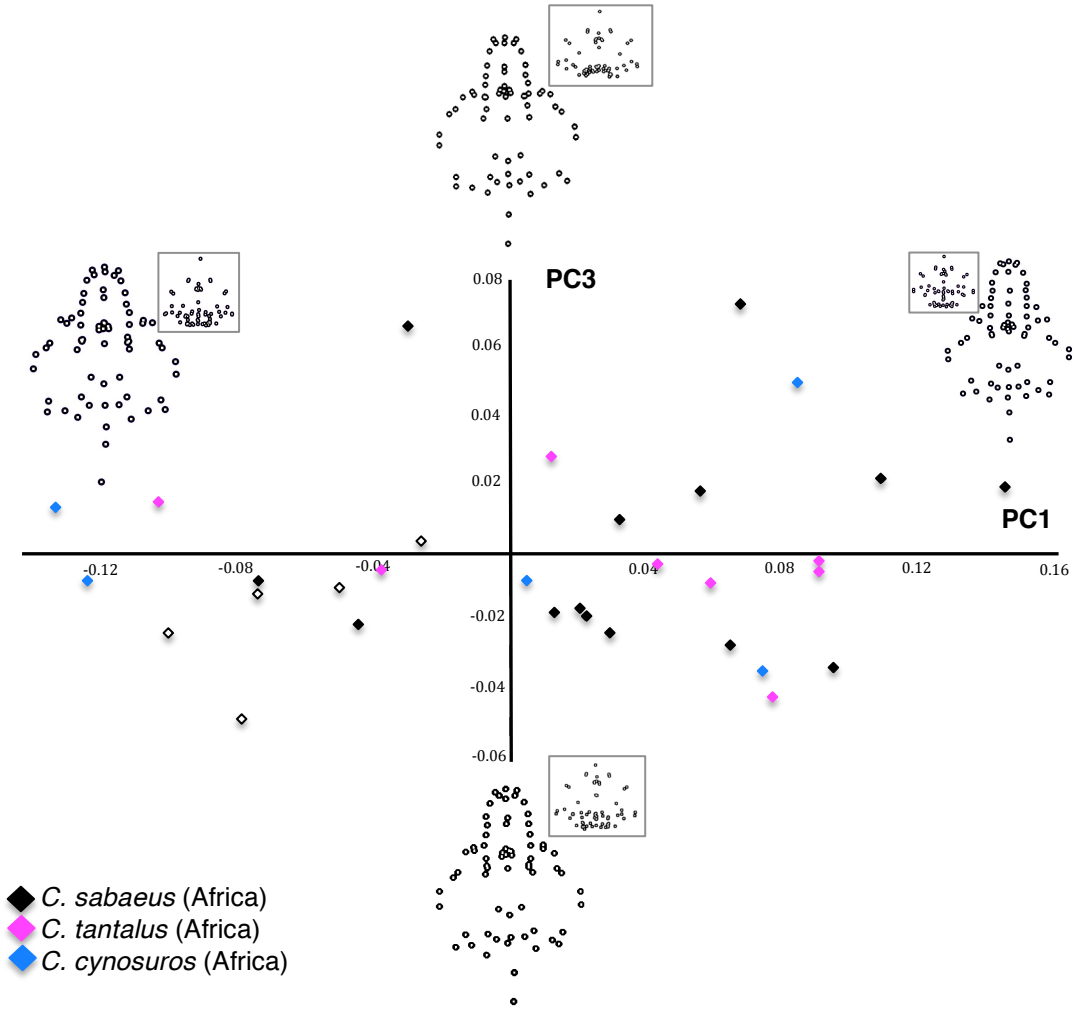

Figure 3.8.2. Plot of PC 1 vs. PC 3 for African male cranial shape from African-sourced *C. sabaeus* (black), *C. tantalus* (pink) and *C. cynosuros* (blue) samples, incorporating size and shape (after Procrustes superimposition). Male samples are represented by shaded boxes and females represented by unshaded boxes. Point-based images illustrating the changes in basicranial and maxilla-facial (inset) shape across each axis are indicated at the ends of each axis. Point image scales: ventral view 1 : 5, dorsal view 1 : 7.

The results from the cluster analysis (Fig 3.8) show clear groupings, in which Caribbean and African samples are separated within the dendrogram. Samples from Barbados are distinct from the Nevis and St Kitts samples but do share clear similarities with African samples from across the *C. sabaeus*

and *C. tantalus* range, especially those from the areas of Sierra Leone, Cameroon and Gambia. Other areas of the dendrogram show a close affinity between samples from St Kitts and Nevis but again, these also show a close relationship with *C. sabaeus* and *C. tantalus* samples. When compared with the Barbados samples, the St Kitts and Nevis samples appear to show a closer relationship with African-originating *C. cynosuros* samples, from the Zimbabwe and Zambia region. Although Nevis and St Kitts samples are distinct from the Barbados samples, they show further distinctions from each other. The cophenetic coefficient for the dendrogram was 0.98.

In order to thoroughly test for differences between all species/populations, a one-way ANOVA was used to assess whether there are statistically distinct populations on the three Caribbean islands. The results showed that these three populations are significantly distinct from one another at a <0.01 level ANOVA ($F(2,87) = 5.7906$, $P = 0.0044$) (Table 3.8). When looking at whether the mainland African species are statistically distinguishable from one another, no significant difference was found ANOVA ($F(2,87) = 0.8507$, $P = 0.4307$) at a <0.05 level (Table 3.9). Repeats for the African species were made using PC2-PC5 as the dependant variable but all results for African samples were not significant.

Table 3.8. Results of one-way ANOVA between the Caribbean island populations. Within this test, PC1 was used as the dependant variable. Showing statistically distinct populations (at a <0.01 level) on the three islands.

	Sum of squares	Df	Mean square	F	P value
Between groups	0.015	2	0.0075	5.7906	0.0044
Within groups	0.113	87	0.0013		
Total	0.1281	89			

Table 3.9. Results of one-way ANOVA between the mainland African species. Within this test, PC1 was used as the dependant variable. Showing that African species are not statistically distinguishable from each other (at a <0.05 level). Repeats using PC2-PC5 revealed similar, non-significant results.

	Sum of squares	df	Mean square	F	P value
Between groups	0.0077	2	0.0038	0.8507	0.4307
Within groups	0.3928	87	0.0045		
Total	0.4005	89			

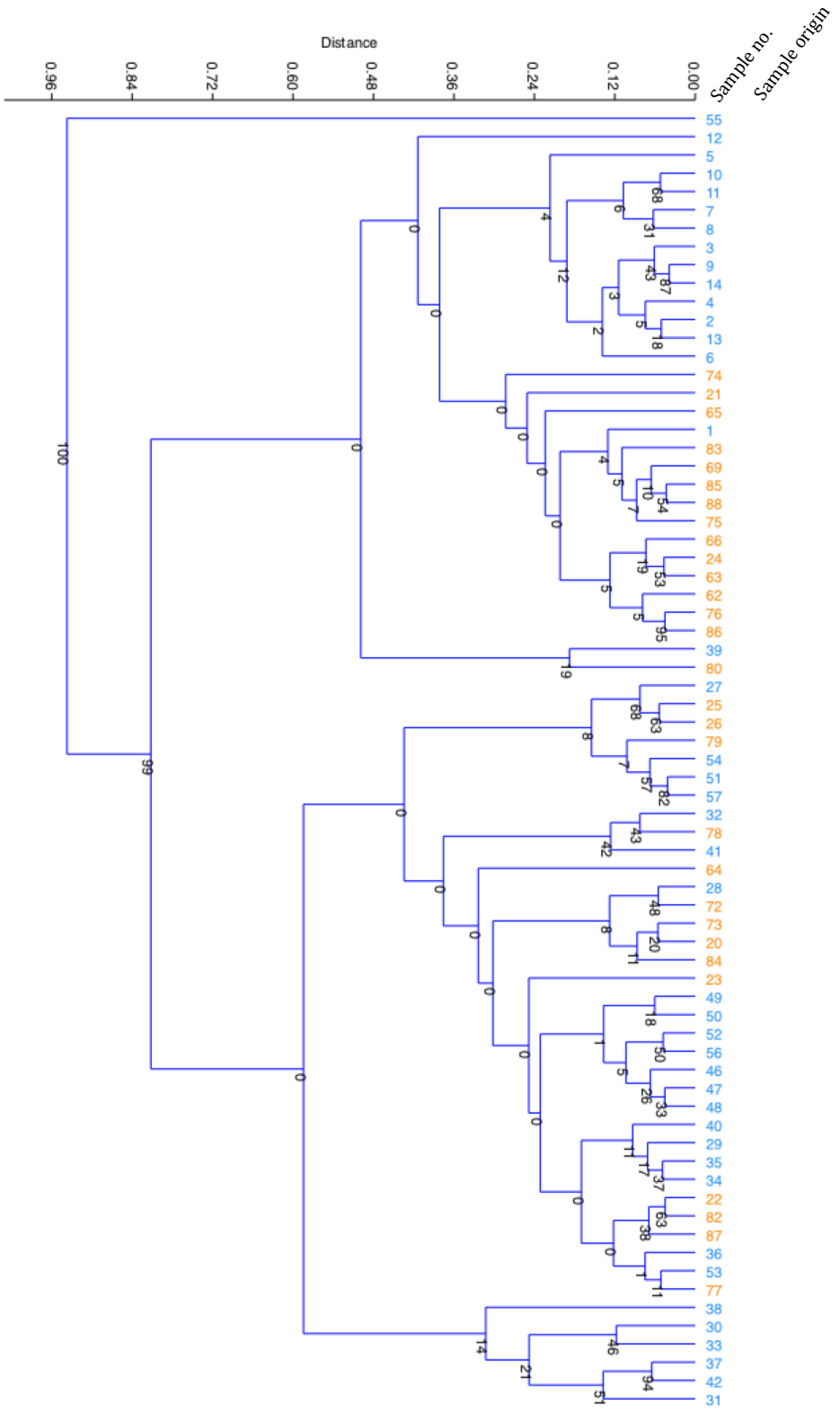

Figure 3.8. Dendrogram based on Ward's cluster analysis (PAST software 3.12) using cranial coordinates after General Procrustes Analysis (GPA). Bootstrapping of analysis was performed with 5000 replicates. 'Sample no.' shows the broader origin of the sample: blue – Caribbean samples; orange – African samples. Specific colours are used to identify specific countries of sample origin.

The variation in shape was further examined by exploring the shape space and the size-shape space of all samples (pooling sexes and species). A PCA of shape coordinates was performed and scatterplots of the main axes of variation were examined using a matrix of shape coordinates and log-transformed centroid size (Cardini and Elton, 2008), indicating a positive allometric relationship overall between cranial shape and size. A Spearman's rank-order correlation was run to determine the relationship between PC1 and centroid size. There was a positive correlation (Fig. 3.9), which, using a Spearman's correlation, was statistically significant at a 0.05 level (r_2 = .616, p = 0.005).

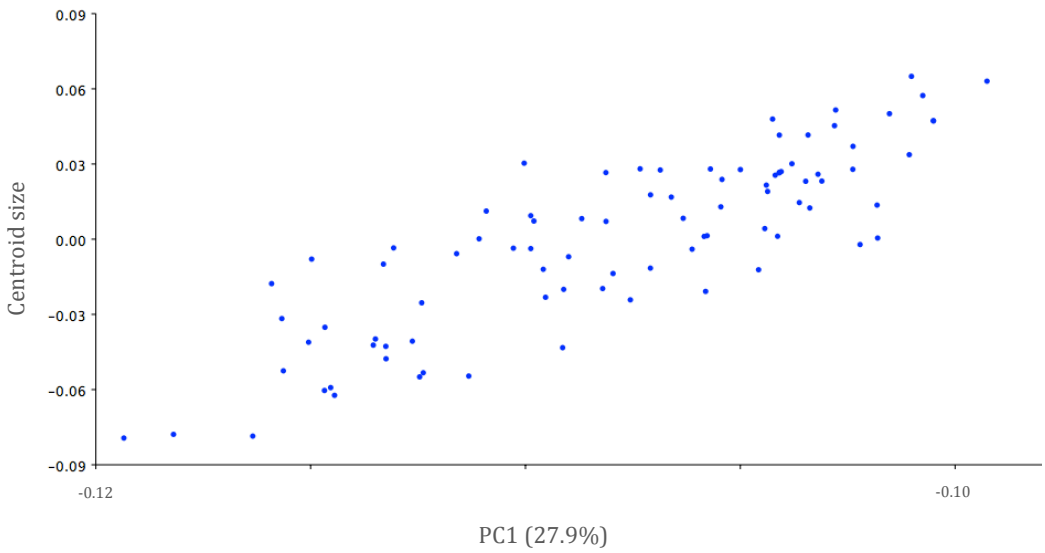

Figure 3.9. Scatterplots of PC1 against centroid size, for shape variables of all species with pooled sexes. Percentages explained in parentheses.

In looking at fluctuating (or bilateral) asymmetry, there was an observable difference between the left and right sides of Caribbean monkey crania. Through pooling all Caribbean crania and analysing the data by Procrustes ANOVA (Table 3.10), results showed that variation in

symmetric shape among individuals accounts for the largest portion of the total variation.

Table 3.10. Procrustes ANOVA for the Caribbean *Chlorocebus* crania. Sums of squares (SS) and mean squares (MS) are in dimensionless units of Procrustes distance. This analysis adds the sum of squares over landmarks and coordinates and assumes that all landmarks have the same amount of isotopic variation.

	df	SS	MS	F	P (parametric)
Individual	103	0.12668	0.00012	3.03	<0.0001
Reflection	99	0.00891	0.00010	2.42	<0.0001
Individual x Reflection	91	0.00369	0.00041	0.39	1.0000
Measurement error	194	0.02040	0.00011	1.73	<0.0001

Variation of left-right means among individuals and fluctuating asymmetry are statistically significant. The results for Procrustes ANOVA on individuals confirm that there are significant differences between them ($p \leq 0.0001$, df = 103, $F = 3.03$) and that there is fluctuating asymmetry in Caribbean *Chlorocebus* crania. Although there was also a significant result for pooled African *Chlorocebus* skulls, the difference was not as apparent ($p \leq 0.0001$, df = 127, $F = 2.36$). In considering the potential impacts caused by measurement error, there was no reason to expect the measurement error to be different between African and Caribbean monkeys.

3.4 Discussion

This study aimed to use *Chlorocebus* as a model for interspecific variation in mammals through the assessment of cranial shape variance. Through the use of 3D geometric morphometrics, it is clear that when looking at *Chlorocebus* monkeys from central and western Africa (representing *C. sabaeus*, *C. tantalus* and *C. cynosuros*), clear differences in terms of cranial shape are not present, although there does appear to be a tendency for *C. cynosuros* crania to be more highly vaulted and have slightly broader muzzles. This is in contrast to the three Caribbean *Chlorocebus* populations, where cranial shape shows both a noticeable difference across the three islands and appears to show some level of distinction between Caribbean and African crania. The main observable shape differences between the Caribbean crania are narrow zygomatic arches in Nevis crania, broad zygomatic arches in Barbados crania and very broad arches in St Kitts crania. The St Kitts crania also have a wider facial region than both the other Caribbean crania and those from western Africa. Although limited behavioural studies have been conducted (Balau *et al*. 1980; Horrocks, 1982; Boulton *et al*. 1996), further, more comprehensive, investigations into the possible effects of differing diets between the islands would resolve whether this finding is due to feeding behaviour. In creating the dendrogram, it became evident that while there is clear (and strongly supported) clustering between many of the Caribbean and African samples, there are also clusters of Caribbean

samples, showing separation between these three groups. The Barbados crania are the most distinct of the Caribbean populations. This finding supports the idea that the Nevis population was started from translocated St Kitts animals. Additionally, whereas the Barbados samples are most closely grouped with *C. sabaeus* samples from Sierra Leone and Gambia and Cameroon samples of *C. tantalus*, both the Nevis and St Kitts crania show close ties with Zimbabwe and Zambia, as well as other African *C. tantalus* and *C. sabaeus*. From this dendrogram, it would appear that while all three Caribbean populations share ties with the same African populations, those from Barbados show no similarities to those from southern Africa. Additionally, the Caribbean populations each show an observable level of morphological homogeny.

The study addressed the following: 1) identified major patterns in cranial shape across the western and central African *Chlorocebus* taxa; 2) identified whether any observed cranial morphometric divisions correspond with existing current African *Chlorocebus* taxonomy and; 3) whether these findings help resolve the African *Chlorocebus* taxonomic ambiguity. Focus on the Caribbean populations of *Chlorocebus* monkeys tested whether there are quantifiable differences 4) between these three insular populations and; 5) if there is any correlation between these Caribbean monkeys and any of the African *Chlorocebus* taxa.

In looking at (1) cranial shape across African *Chlorocebus*, this study found that there is no definite pattern in cranial shape differences between the three western species when potential effects of allometric scaling had been removed. In these African species, morphological variation across such a broad geographic range appears to be small and gradual (i.e. clinal), which is likely driven by relatively small changes across the environment. *C. cynosuros* crania do appear to be more highly domed than the others, with *C. sabaeus* crania having wider and shorted muzzles. Samples from *C. sabaeus* and *C. cynosuros* appear broadly distinct from one another, with *C. tantalus* samples falling predominantly between the two (Fig 3.6.1 and Fig 3.6.2). This seemingly indistinct pattern would appear to reflect the natural distribution of the three species, where there are areas of overlap between *C. sabaeus* and *C. tantalus and between C. tantalus* and *C. cynosuros* but not between *C. sabaeus* and *C. cynosuros.* In terms of looking at the congruence of these results with previous findings (2), this study corresponds with previous research where it was observed that cranial morphological variation (in terms of size) in African *Chlorocebus* is seen mainly at the extremes of their longitudinal distribution, with the three western species being significantly larger than the three eastern species (Elton *et al*. 2010) and does not follow a clear pattern of latitudinal differentiation. Whilst there are some observable differences in cranial shape in these three African species, there are no definitively clear distinctions. This pattern of no clear differentiation may be due to the fact that these species inhabit broadly similar habitats. As

primary production is higher in western and central Africa than it is on the continent's eastern side and it may be the case that because the three African species focused on in this study face broadly similar environmental, intra- and inter-specific influences, they may have arrived at an optimal shape shared across these species, which accounts for a lack of clear differences across the species.

In trying to resolve African *Chlorocebus* taxonomic ambiguity (3), it is apparent that neither cranial size nor shape is able to resolve the finer points of this problem-strewn taxon any further than major East-West divides. As is often the case with geometric morphometric studies, there was a limitation of available and useable samples and the question arises as to whether this had an effect on the results. In future analyses, the use of smaller landmark sets would potentially help maximise these limited specimen numbers. Being spread across numerous collections in multiple continents, a lack of availability for some species, poor museum accessioning practices and significant damage to crania (drastically limiting the number of specimens available with a full set of accessible landmarks) all contributed to limiting the sample sizes in this study. However, estimates of primate cranial shape variance in small samples tend to remain accurate and precise (Cardini and Elton, 2007) and even in samples of ten individuals, 95% of shape variances are within a range of 0.75-1.25 times the observed from much larger sample sizes. Providing individuals from these small samples are representative (from across most

of the distribution range of the species or population), such relatively small samples are still able to provide accurate estimates of the magnitude of shape variation across populations of cercopithecine primates. As a result, whilst larger samples may have further elucidated any patterns seen in the shape variance of the crania, the small samples in this study are reliable in showing these patterns in the first instance. Despite 3D geometric morphometrics being a powerful tool in using shape to elucidate primate taxonomy and phylogeny in some taxa (Frost *et al*. 2003; Baab, 2008; Gilbert, 2010) and that when looking at shape variance, results are not affected when small samples are used, its use in resolving *Chlorocebus* taxonomy is not effectual, probably as a result of the widespread and often overlapping distribution of the *Chlorocebus* taxa.

Focus on the Caribbean populations of *Chlorocebus* monkeys tested whether there are quantifiable differences 4) between these three insular populations and found that there are clear distinctions between the Caribbean *Chlorocebus* populations. In looking at males, crania from St Kitts have a short muzzle, high-domed crania and broad zygomatic arches. Crania from the nearby Nevis are broadly similar but differ in that they have a narrower, longer muzzle and look more prognathic in their overall appearance. Samples from Barbados males do not overlap with those from Nevis or St Kitts (Fig. 3.5.1 and Fig 3.5.2). By comparison, samples from St Kitts and Nevis are largely mixed in with one another. This complies with the natural history of these two groups, where the

Nevis population was a subsequent introduction from the already-established population on St Kitts (Poirier, 1972; McGuire, 1974; Denham, 1987), which lies less than 2 km away. The amount of variation within these three populations also appears to differ. Both Nevis and St Kitts are characterised by a considerable degree in variation in shape when compared to crania from Barbados. With adequate sample sizes and ensuring that all samples were from adult animals, these differences maybe attributable to as yet unknown differences in either environmental or intraspecific influences between the Barbados population and those from Nevis and St Kitts. In looking at female Caribbean cranial shape, although the sample sizes were relatively small, the PCA results showed that there were clear differences between the three island populations (Fig. 3.6.1 and Fig. 3.6.2). Again, crania from St Kitts and Nevis are broadly similar to one another, both possessing a broad muzzle, with very wide zygomatic arches. Crania from Barbados have a narrower muzzle and narrower zygomatic arches, but have a much longer muzzle and a cranium that is less vaulted. In this PCA, there was also less variance seen in the Barbados population when compared to the other two populations. Such results in female samples may result from environmental differences, such as varying diets. Further investigation using a larger sample size may strengthen these findings. One-way ANOVA analysis demonstrated that there are statistically significant shape differences between the three Caribbean island populations. Additionally, Procrustes ANOVA testing showed that overall, Caribbean crania display a

significant level of bilateral asymmetry (higher than that seen in African crania). As this developmental phenomenon is an accepted measure of individual capability that can be directly related to developmental stability (Maestri et *al*. 2015), with observable asymmetry being associated with increased levels of genetic and possible environmental stress, it seems apparent that the Caribbean *Chlorocebus* monkeys are subject to some increased level of developmental stress. Although the selective pressure supplied by predators is greatly reduced within the Caribbean, it is possible that (despite the availability of high-calorific fruit crops) the large populations of monkeys on the islands are causing a heightened level of intraspecific competition (pers. obs.), creating a level of developmental instability.

Finally, in looking at 5) whether there is any correlation between the shape of the Caribbean monkeys and any of the African *Chlorocebus* taxa, whilst there is no major pattern of differentiation between the western and central African *Chlorocebus* crania in terms of shape, the area of greatest overlap is shown between African *Chlorocebus* and those Caribbean samples from Nevis and St Kitts. Because of the clinal nature of the pattern in shape diversity in *Chlorocebus* crania, it is not possible to give a definite answer as to where the Caribbean monkeys originated but from the PCA results, it appears that generally, Caribbean *Chlorocebus* crania are most synonymous with those from African populations of *C. sabaeus* but also share some moderate level of similarity with both *C. tantalus* and *C.*

cynosuros. This could be due to either there being multiple sources of origin for the Caribbean monkeys from across the distribution of the three African species discussed here, or because 3D geometric morphometric techniques cannot adequately distinguish such small clinal differences in the broader *Chlorocebus* taxon. In broadly comparing mainland African with Caribbean *Chlorocebus* monkeys (Fig 3.8. and Fig. 3.9), the Caribbean crania are significantly more distinct from each other than the nominal species on mainland Africa are from one another.

Research from the 1950's used univariate analysis to show that *Chlorocebus* monkeys from St Kitts showed both greater cranial dimensions and less variability in these cranial dimensions when compared to African *C. sabaeus* samples (Ashton and Zuckerman, 1951a; Ashton and Zuckerman, 1951b). The research described here supports these earlier findings through multivariate analysis using 3D geometric morphometrics and in addition to this, shows for the first time that crania from the Nevis and Barbados populations both show a similar pattern and some level of morphological difference from the St Kitts population. Although the loadings from these results are supported by relatively low eigenvalues, meaning that the observed differences cannot be predominantly contributed to one or two factors alone, this sort of pattern might be expected from an African taxon which is known for it's lack of defining features and a series of island populations that have only just started on their own evolutionary trajectory and have not had sufficient

time in which to possess any one clearly defining feature. Overall, Caribbean crania have a longer, narrower muzzle than those from African *Chlorocebus* and are much less vaulted, whereas the African crania tend to appear more domed and have shorter, broader muzzles. It is commonly reported that the Caribbean monkeys originated solely from African populations of *C. sabaeus* (Ashton *et al*. 1979; Van der Kuyl *et al*. 1996; Haus *et al*. 2003) but the results from this study do not support that. Further research would help elucidate whether differences in Caribbean *Chlorocebus* cranial shape are due to either founder effect or as a result from having multiple African origins. Additionally, because the number of principle components extracted was low (much lower than the number of specimens), a high level of redundancy in the landmarks was probable. Further work would be able to resolve whether this was due to the fact landmarks were scored from both sides of the crania. Additionally, because landmarks from both sides of the crania were recorded, the possibility of cranial asymmetry could be explored further.

3.5 Conclusion

African *Chlorocebus* monkeys are a complex group found through much of sub-Saharan Africa, where different species are often contiguous with one another and hybridisation is possible. In terms of cranial shape, no clearly distinctive patterns of division were found in the three African species of *Chlorocebus* studied here (*C. sabaeus*, *C. tantalus* and *C. cynosuros*) but instead form a clinal pattern of variation, although *C. cynosuros* crania do appear to be more highly domed than either *C. sabaeus* or *C. tantalus*. When looking at the Caribbean populations of *Chlorocebus* monkeys, although there was some obvious overlap, PCA analysis showed that the crania from the three island populations were not only noticeably distinct from the African species' crania but were distinct from one another. Such broad differences between the Caribbean populations was (highly) statistically supported by one-way ANOVA analysis. Broadly, the Caribbean crania had longer, narrower muzzles than African crania and are less vaulted. The crania from St Kitts and Nevis were of similar shape to one another, reflecting their shared Caribbean origin (on St Kitts) but differed from those from Barbados in that they are more vaulted and have a slightly shorter muzzle. Whereas changes in size in primates appear to be the 'line of least evolutionary resistance' (Marriog and Cheverud, 2005), changes in shape appear to be more conservative, especially in rapidly changing environments (Elton *et al*. 2010). This may explain why there is little variation in terms of shape in the three western species of African

Chlorocebus but more marked differences in terms of size and that after just approximately 400 years of separation, the Caribbean populations are showing more noticeable differences in terms of cranial shape, which may reflect shape-based adaptations due to swift and significant changes in selective pressures within and across these insular ecosystems, such as increased intra-specific competition (Sade and Hildrech, 1965) or changes in their diets. Supporting this idea is the finding that there is a higher level (than that seen in mainland African *Chlorocebus* crania) of bilateral asymmetry in Caribbean *Chlorocebus* crania. Being used as an indicator for developmental stress (due to genetic or environmental influences), including stressed marginal habitats, which are commonly found in regions outside the environmental optimum of a species (Parsons, 1982), it may be that the Caribbean *Chlorocebus* monkeys are experiencing more developmental stress (Maestri et *al*. 2015), which may in turn be accelerating cranial shape change.

Further study into the exact nature of this shape change may elucidate what these influencing factors are and how the Caribbean monkeys have started to functionally adapt to suit these new insular environments. This study not only supports recent findings in that the African *Chlorocebus* taxa are not subject to cranial differences along a latitudinal scale but more importantly for the first time finds that the Caribbean *Chlorocebus* crania are not only different from their African counterparts but are different from each other within the Caribbean and show a level of

distinctiveness higher than that observed between western African species.

Chapter 4

Investigating a population-level cranial deformity in introduced island-living *Chlorocebus* monkeys.

4.1 Introduction

Within the fields of anthropology and zoology, the skeleton and especially the skull represents an important source of anatomical data. The analysis of skeletal material at a macroscopic level is important to diverse areas of research, including biological anthropology (Matsuzawa *et al.* 1990; Aiello and Dean, 2002; McFarlin *et al*. 2008; Bolter & Zihlman, 2012), evolutionary development (Zihlman and Tanner, 1978; Fleagle *et al*. 2010; Gilbert, 2010; Arias-Mertorell *et al.* 2014; Zihlman *et al*. 2004), captive husbandry (Bolter and Zihlman, 2006; Druelle and Berillon, 2014), bioarchaeology (Hesse and Wapnish, 1985; Roberts and Mays, 2011) and applied conservation (deOliveira *et al*. 2008; Hansford *et al*. 2012). In many of these instances, the presence of specific trauma and pathological conditions within the skull can further our understanding of veterinary management (Pérez *et al*. 2004), captive animal welfare (Farrell *et al*. 2015), predator-prey interactions (Berger and Clarke, 1995) and the evolutionary ecology of specific pathologies and pathogens (Sassoon *et al*. 2012).

Several diseases, such as diabetes, craniosynostosis and syphilis, have an increased associated risk of mineral metabolism and cranial pathology. Excess bone formation, a reduction of bone mineral density, decreased skeletal mass, alternating linear growth and delays in fracture healing have all been linked to the presence of diseases (Abbassy *et al.* 2008) and

whilst most research into cranial pathology focuses on humans, there is limited research into skeletal cranio-pathology in non-human animals (Gorlin, 1951; Johansson *et al.* 2002; Pérez *et al.* 2004; Sonne *et al.* 2009; Biebach and Keller, 2012; Murphy *et al.* 2013; Johnson *et al.* 2014; Slon *et al.* 2014), and less still on non-human primates (Ruch, 1959; Barker and Herbert, 1972; Jurmain, 2000; Mitja *et al.* 2013).

Whilst diseases that affect the skeleton are commonly seen in captive primates (Ruch, 1959; Barker and Herbert, 1972; Farrell *et al.* 2015) such pathologies are only rarely seen at a population level in wild-living primates (Schultz, 1969; Jurmain, 1989). However, a specific cranial pathology was observed in a population of *Chlorocebus* monkeys (*Chlorocebus sabaeus*) living in the Caribbean. Transported in slave ships and originally intended as pets, monkeys were taken from Africa to the Caribbean between 1627-1807 (Denham, 1987), where they were introduced to the islands of St Kitts, Nevis and Barbados. Whilst historical reports state that the monkeys were taken from multiple ports in Senegal and Gambia, Gabon and Nigeria, and as far south as Angola and South Africa, and were transported in their hundreds (Denham, 1987; McGuire, 1974), recent molecular evidence proposes that the Caribbean populations are phylogenetically clustered, appearing to represent populations which stem from a single (or limited) source population (Brown *et al.* 2013). After what is assumed to have started as several small, captive populations, the monkeys were either released or escaped and were recorded as being

feral and in large numbers by 1682 (Denham, 1987). Since this time, there have been large numbers of monkeys on each of the three islands, with conservative estimates of over 12,000 monkeys on St Kitts (Coppinger and Maguire, 1980) and over 25,000 on Barbados (Poirier, 1972).With some research proposing that the broader genetic lineages represented by founder monkeys imported from multiple parts of Africa may have been lost (Van der Kuyl *et al*. 1996), the effects of inbreeding may be apparent within the Caribbean population. During data collection for morphometric analysis, it was observed that a gross pathological malformation was present in numerous adult crania. After some initial investigation, it was noted that the pathology was not seen in any other species of primate but only in *Chlorocebus* specimens and that in particular, was found to only be present only in Caribbean specimens. The aim of the present study is to describe and investigate the gross pathological findings and aetiology associated with the cranial pathology seen in the wild-living Caribbean populations of *Chlorocebus* monkeys and to investigate whether any causative factors can be associated with this pathology.

4.2 Methods

4.2.1 Samples

A macroscopic examination of 336 *Chlorocebus* skulls was performed (Table 4.1). Each museum skull had been labelled previously with catalogue number, sex, collection location and date. Samples collected directly from the Caribbean (Nevis and Barbados) were taken from freshly-killed animals, which had been euthanised as part of an ongoing, government-led culling programme. All research on this project complied with ethical protocols and procedures set out by University College London, the Zoological Society of London, the Barbados Primate Research Centre and Wildlife Reserve and the Nevis Ministry of

Table 4.1. Showing adult skulls used for macroscopic examination, with numbers of skulls assessed and the number of specimens displaying pathology. * denotes skulls from the six African *Chlorocebus* species: *Chlorocebus sabaeus*, *C. tantalus*, *C. cynosuros*, *C. aethiops*, *C. pygerythrus* and *C. djamdjamensis*.

Place of origin	Collection / source	No. skulls	No. skulls with pathology
St Kitts	Royal College of Surgeons (RCS UK)	79	13
Nevis	Nevis – collected *in situ*	21	1
Barbados	Barbados – collected *in situ*	36	0
Africa*	RCS and Nat. Hist. Museum, London (UK)	200	0

Agriculture, Marine Resources and Cooperatives. All Barbados monkeys were originally wild-caught but had spent at least 12 months in a biomedical facility, prior to death. Age classes were separated into 'infant/juvenile' and 'adult', determined by the full dental eruption of M4 in adults. Additionally, adult sutures showed partial or complete obliteration.

4.2.2 Differential diagnosis

A differential diagnosis was used in order to try to identify the described *Chlorocebus* pathology. This approach aims to identify a condition where multiple alternative pathologies are possible (Barnes, 1994). A differential diagnosis works by eliminating conditions that do not fit the criteria for a full diagnosis, leaving fewer (or just one) potential conditions. A combination of symptoms, life history (of the affected individual) and medical knowledge are used to aid the diagnostician in clarifying their epistemic confidence. In order to develop a thorough differential diagnosis, a technique known as a 'surgical sieve' was used (Turmezi, 2009). This process creates a structured examination, looking at potential conditions from a broad range of specific epidemiological backgrounds in a clear and structured manner. Within this study, pathologies from the following backgrounds were used: Vascular (Circulatory), Infectious, Trauma, Metabolic, Idiopathic, Neoplastic, Degenerative, and Developmental (using the standardised surgical mnemonic 'VITMINDD'). Macromorphological osteological analysis was used to determine the presence of the observed

pathology in skulls. Radiographic and Micro-CT imaging were then used to investigate whether further symptoms existed.

4.2.3 Macroscopic analysis

Digital callipers (Moore and Wright Digitronic 110 Series; resolution: 0.01-300mm) were used to undertake macroscopic examination in order to allow for a differential diagnosis to be performed. Pathological symptoms (that were non-injurious in their nature) were recorded as present or absent and measurements were taken when accessory bones were present and for foramen magnum measurements (Table 4.2). Grading of the pathology was not possible, as it was either present or absent – no intermediate examples were found, for example. Only the dimensions of the foramen magnum and whether a supernumerary bone in the frontal bone was present or absent varied. Fourteen skulls were found to possess signs of the pathology. Once skulls showing pathologies had been identified, the same number of unaffected adult skulls (14) was randomly selected for foramen magnum measurements. Many of the skulls were damaged in the posterior basicranial region, which is associated with poor historical specimen preparation and handling. In these cases, although it was not always possible to measure the foramen magnum, the malformation was still clearly visible. To measure the foramen magnum, standardised morphometric landmarks (the distance between the opisthion and the basion) were used in unaffected skulls (Schady *et al.* 1987) and for affected individuals, the length between the proximal and distal points

was measured (Fig 4.1). For all skulls, the distance between the most lateral and medial points of the foramen magnum were measured.

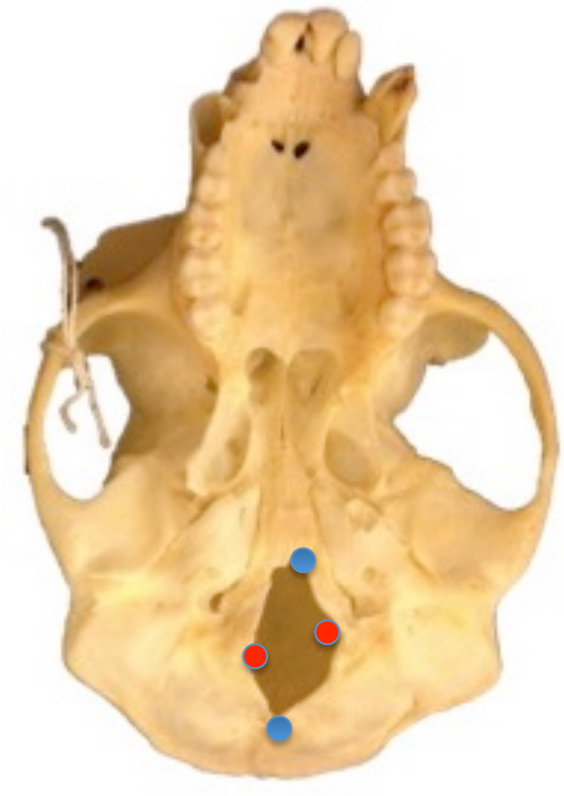

Figure 4.1. Foramen magnum measurements were taken between two standard cranial landmarks: the opisthion and basion (in blue) and between the most lateral points of the foramen magnum on either side. All measurements were taken in mm.

4.2.4 Radiographic imaging

To obtain high-resolution radiographic images of the skulls, a Faxitron Microfocus Imaging System (Typ. 30kv for 120 second exposure; Film: Fuji IX 80) was used to generate exposures. Cassettes were not used, as

exposure was made on bare film under safelight conditions. All processing was manual, in Tetanol Developer.

4.2.5 Computed Tomography (CT) assessment

Due to constraints with access and timing, it was only possible to CT scan two skulls. Two specimens were selected for CT scanning analysis, in order to compare gross pathology and to take generalized measurements. One pathological adult male and one unaffected adult male skull were used. Each specimen was scanned on an i-CAT cone beam computed tomography (CBCT) unit at the Natural History Museum, London; all scans took place on the same day and on the same machine, following standardised protocols (Farrell *et al.* 2015). The unit was calibrated to the Hi-Fi setting (90Kv, 214.20mAs) to obtain the maximum radiation dosage. This was necessary to provide the clearest images and to best differentiate between different densities. Axial slices were taken at 0.25mm increments, with an imaging window of 640x640mm, where the total field of view was 16cm. DICOM images were extracted from the scanning unit and imported into dedicated CT/CBCT viewing software, VGStudioMax 2.2 (64bit), for virtual reconstruction. The software reformats the original axial slices into coronal and sagittal slices, and allows for the creation and use of virtual 3D models.

Once scanned, measurements were taken from a standardised sectioned image. Images were sectioned (in a coronal plane) at the level of the most

anterior point of the zygo-matico frontal suture (at the standardised ectochonchion craniometrics landmark). Six measurements (to accommodate bone thickness) were taken from the left and right sides of the skull (Fig 4.2) to assess areas of potential difference in the facial (measurements A-C) and parietal or cranial (measurements D-F) regions (Table 4.2).

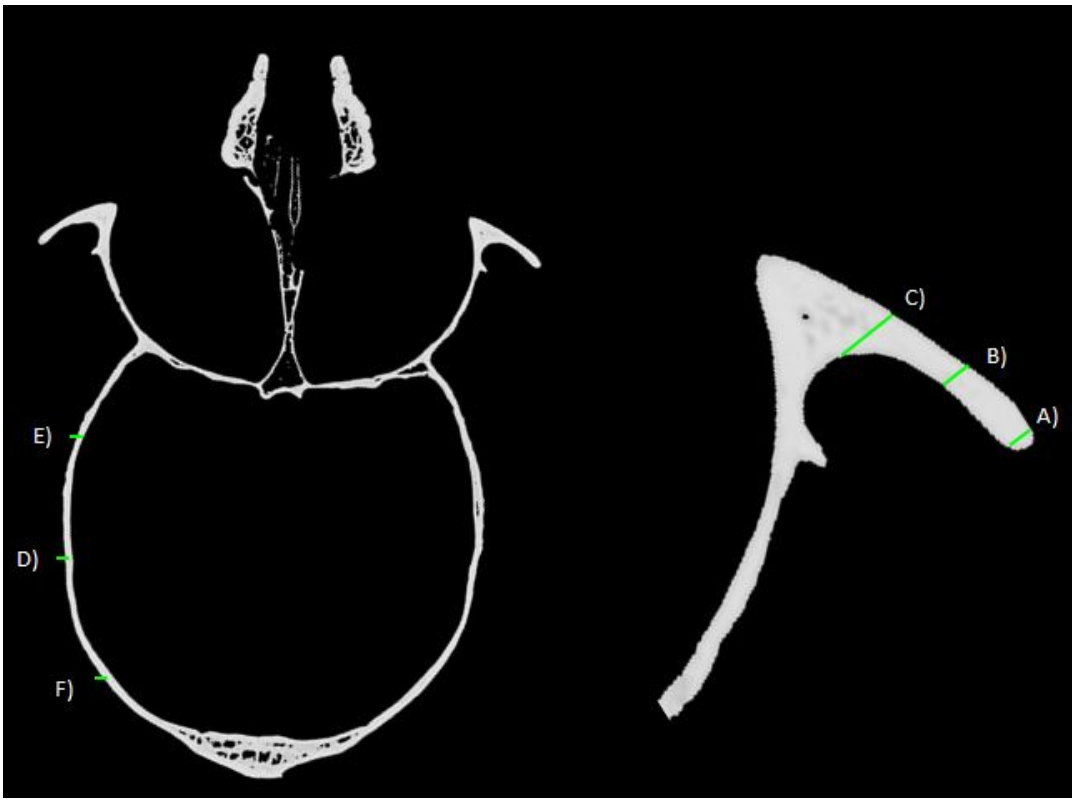

Figure 4.2. Measurements taken from skulls (from both left and right sides) to assess for facial (A-C) and cranial (D-F) differences between pathological and unaffected skulls. Right zygomatic region (around the zygo-matico frontal suture) is enlarged.

4.2.6 Statistical analysis

In analysing the data to test the difference in results between samples, an unpaired Student T-Test was used, which looks at scale/ordinal

dependent-variable data in categories distinguished by the independent variable (Hawkins, 2005), where each sample must be approximately normally distributed and the variance of the two samples must be similar (Pallant, 2007).

Table 4.2. Measurements taken from sectioned MicroCT images; from one pathological adult male skull and one unaffected adult male skull. Overall, the pathological skull appears to show cranial bone thinning around the parietal region (at the area of greatest curvature and around the posterior part of the calvarium). All measurements given in mm.

	Pathological skull		Unaffected skull	
	Left	Right	Left	Right
A) Edge of zygomatic	1.07	0.97	1.06	1.00
B) Midpoint of zygomatic	0.76	1.03	1.56	1.60
C) Root of zygomatic	2.10	1.89	2.02	2.41
D) Greatest temporal curvature	1.29	0.94	2.74	3.21
E) Anterior midpoint of temporal	1.05	0.96	0.92	1.08
F) Posterior midpoint of temporal	1.14	0.97	3.30	3.77

4.3 Results

The observed pathology was largely characterised by a drastic lengthening of the foramen magnum (Table 4.3). On average, the

Table 4.3. Showing details of 14 pathological skulls and 14 unaffected skulls. 'Broken' denotes where post mortem damage precludes measurement of the foramen magnum but where the pathology is still visibly present. *foramen magnum; ** frontal supernumerary bone; Δ bony ridging present on the frontal bone between the orbits; NP not present. All measurements are given in mm.

Accession No.	Sex	Source	Country	FM* Pathology	FM length	FM width	SNB** (length x width)
A72.636	M	RCS	St Kitts	Present	Broken	Broken	NP
A72.634	M	RCS	St Kitts	Present	35.8	14.8	2.6 x 1.8 Δ
A72.633	M	RCS	St Kitts	Present	Broken	Broken	2.8 x 2.5 Δ
A72.648	M	RCS	St Kitts	Present	35.3	14.3	2.5 x 2.2 Δ
A72.662	F	RCS	St Kitts	Present	Broken	Broken	NP Δ
A72.652	M	RCS	St Kitts	Present	Broken	Broken	NP Δ
A72.664	M	RCS	St Kitts	Present	Broken	Broken	NP
A72.6692	F	RCS	St Kitts	Present	Broken	Broken	3.6 x 3.2 Δ
A72.672	M	RCS	St Kitts	Present	Broken	Broken	NP
A72.652	M	RCS	St Kitts	Present	Broken	Broken	NP Δ
A72.648	M	RCS	St Kitts	Present	30.5	14.2	NP
A72.646	F	RCS	St Kitts	Present	Broken	Broken	2.8 x 1.9 Δ
A72.645	F	RCS	St Kitts	Present	30.0	12.5	NP
Nev0902	M	Nevis	Nevis	Present	29.2	14.0	NP
A72.62	M	RCS	St Kitts	Absent	13.8	13.8	NP
A72.63	M	RCS	St Kitts	Absent	13.4	13.0	NP
A72.64	M	RCS	St Kitts	Absent	13.2	12.9	NP
A72.68	M	RCS	St Kitts	Absent	13.4	13.2	NP
A72.611	F	RCS	St Kitts	Absent	13.0	12.8	NP
A72.612	F	RCS	St Kitts	Absent	13.2	13.0	NP
A72.613	F	RCS	St Kitts	Absent	13.3	13.1	NP
A72.614	F	RCS	St Kitts	Absent	13.3	12.9	NP
A72.615	M	RCS	St Kitts	Absent	13.2	13.1	NP
A72.616	M	RCS	St Kitts	Absent	13.4	13.0	NP
A72.617	M	RCS	St Kitts	Absent	13.0	12.9	NP
A72.618	M	RCS	St Kitts	Absent	13.1	12.8	NP
A72.619	M	RCS	St Kitts	Absent	13.7	13.5	NP
A72.631	M	RCS	St Kitts	Absent	13.9	13.8	NP

foramen magnum of an unaffected skull has a width of 13.1mm and a length of 13.4mm. Also, in non-affected animals, a clearly delineated diploe is present. It is worth noting here that along with humans and some other non-human primates, *Chlorocebus* monkeys possess diploic cranial bones, where there is a cancellous bone and bone marrow-filled space between bones of the inner and outer tables. In animals affected by the pathology, the foramen magnum has a mean width of 14.0mm and a length of 32.2mm and a great reduction and loss of the diploic space. In each affected skull, the most immediate symptom is an enlargement and distortion of the foramen magnum (Fig. 4.3). Additionally, in 35.7% of

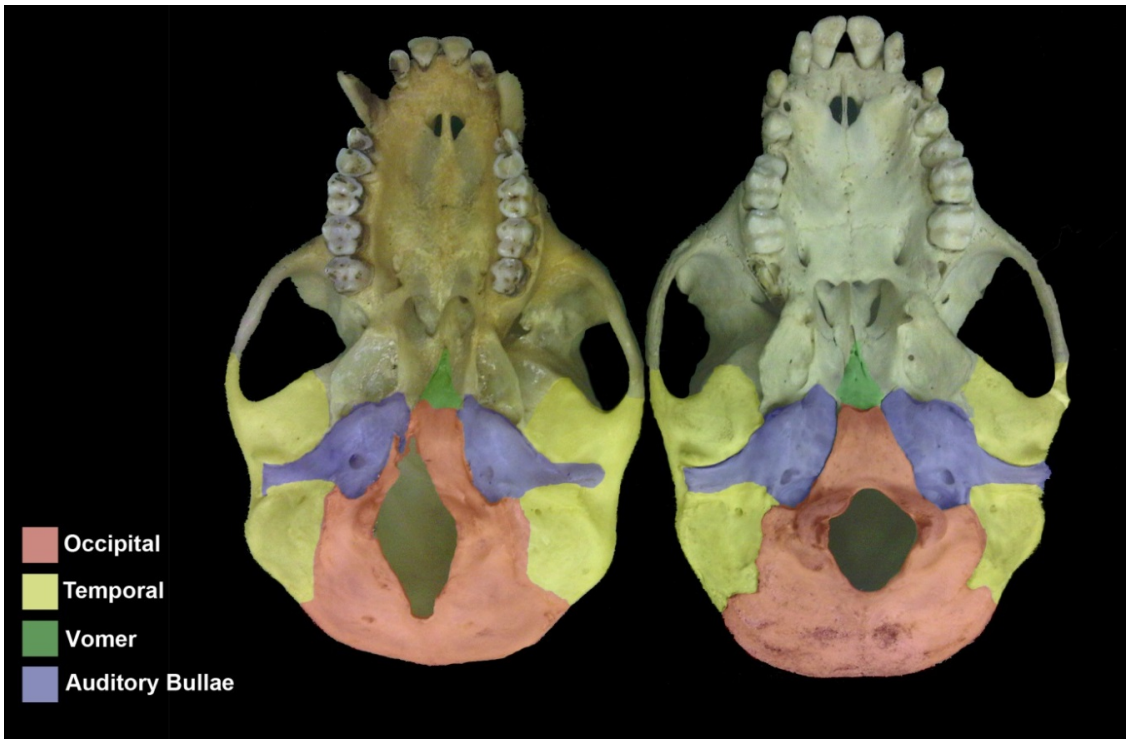

Figure 4.3. Pathological (left) and unaffected (right) skulls, showing marked enlargement and distortion of the foramen magnum. On average, opposing ends (proximal and distal) of the foramen magnum are skewed in opposing directions and when inclusion of the vomer is noted, it skews in the opposite direction to the proximal end of the foramen magnum.

affected specimens, a series of narrow horizontal bony ridges was present on the frontal bone between the orbits. The pathology was observed in 21.4% of the adult monkey population on St Kitts and 4.77% of the adult population on Nevis. No cases of pathology were seen in Barbados or in animals from Africa. In addition to the adult skulls, 76 infant/juvenile skulls were assessed to see whether the pathology was present in earlier life developmental stages. From across the Caribbean populations, 43 (St. Kitts: 32, Nevis: 2, Barbados: 9) skulls were looked at, as well as 33 infant/juvenile African skulls. This pathology was not observed in any infant or juvenile animals within either African specimens or those from the Caribbean. From personal observations, living animals that were later (at necropsy) diagnosed with the pathology did not appear to show any obvious signs of neurological impairment. There is moderate variation in shape of the foramen magnum, with some being very angular and others being moderately rounded but overall, the usually round foramen magnum is distinctly diamond-shaped in its appearance. In some cases, the edges of the foramen magnum extend from the basimidline and as far back as the inion, to the spheno-occipital synchrondosis basilar suture (Fig. 4.4). On average, there is opposing skewing of the distal and proximal ends of the distorted foramen magnum and there is an associated inclusion of the vomer, which tends to be skewed in a direction opposing the proximal aspect of the foramen magnum, indicating a subsequent ‘push’ of the vomer.

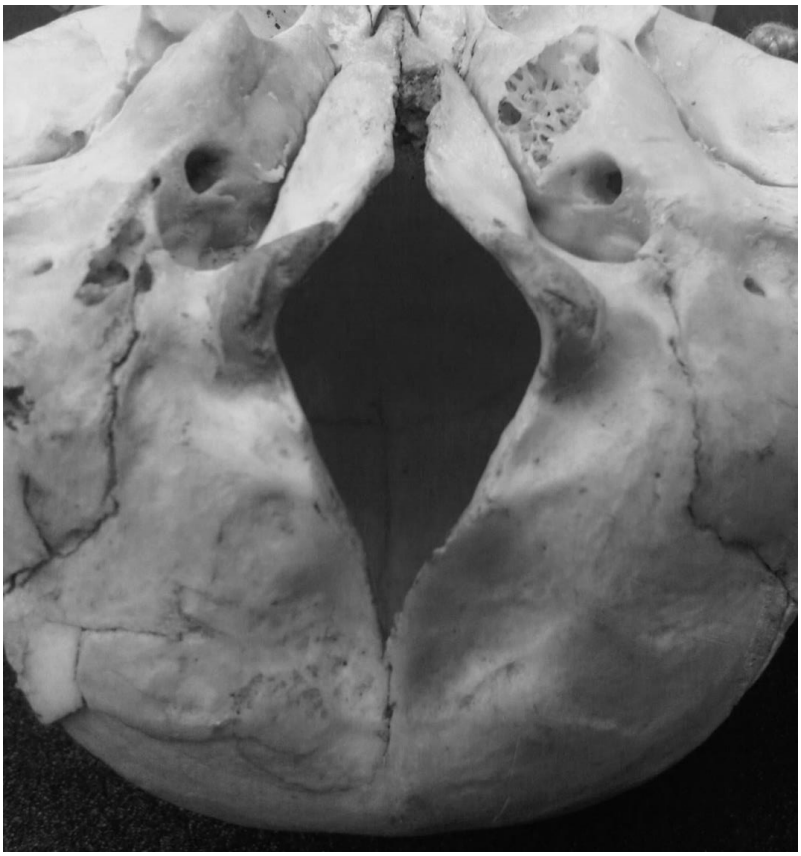

Figure 4.4. In severe cases, the edges of the affected foramen magnum may stretch from the basimidline to the inion, to terminate at the spheno-occipital synchrondosis basilar suture.

When comparing pathological and unaffected skulls, there was a significant difference in the foramen magnum length, $t(8) = 13.35$, $p < 0.00001$, with pathological skulls having much longer foramen magna (Table 4.4).

Table 4.4. Unpaired Student T-Test comparing foramen magna length.

	Mean	N	Std. Dev.	Std. Error Mean
Pair 1:				
Pathological	32.16	5	3.13	1.40
Unaffected	13.36	5	0.30	0.13

In comparing pathological and unaffected skulls, there was no significant difference in foramen magnum width, $t(8) = 1.92$, $p > 0.05$, with pathological and unaffected skulls having foramen magna of similar widths (Table 4.5).

Table 4.5. Unpaired Student T-Test comparing foramen magna width.

	Mean	N	Std .Dev.	Std. Error Mean
Pair 1:				
Pathological	14.0	5	0.87	0.39
Unaffected	13.14	5	0.40	0.18

The bones from affected skulls are thin in their cross-section appearance, which is supported by radiographic and Micro-CT imaging: showing a reduced opacity of the cranial vault when viewed radiographically (Fig. 4.5) and from measurements taken from the Micro-CT images (Fig. 4.2 and Tab. 4.3). In all affected crania, the parietal area especially shows thinning, to the extent that the diploe appears to have been lost. Across the parietal region, the bone from an affected specimen appears to be twice as thin as that from an unaffected individual. From extensive macroscopic identification and radiographic imaging, marked areas of 'copper-beating' (Bourekas and Lanzieri, 1994) can also be seen in all affected skulls (Fig. 4.6), which is diagnostic of an increased intracranial pressure. Diagnostically, areas of the inner table of the calvarium appear

pitted with multiple broad, shallow concavities. Due to specimen availability, only two crania were radiographed but due to the fact that copper beating was visible by macroscopic analysis, this scanning served to explore whether any additional symptoms were apparent through radiograph imaging. This presence of copper-beating was visual by eye when looking through the foramen magnum with the use of a hand lens and was confirmed by a forensic anthropologist (C. Duhig). As intracranial

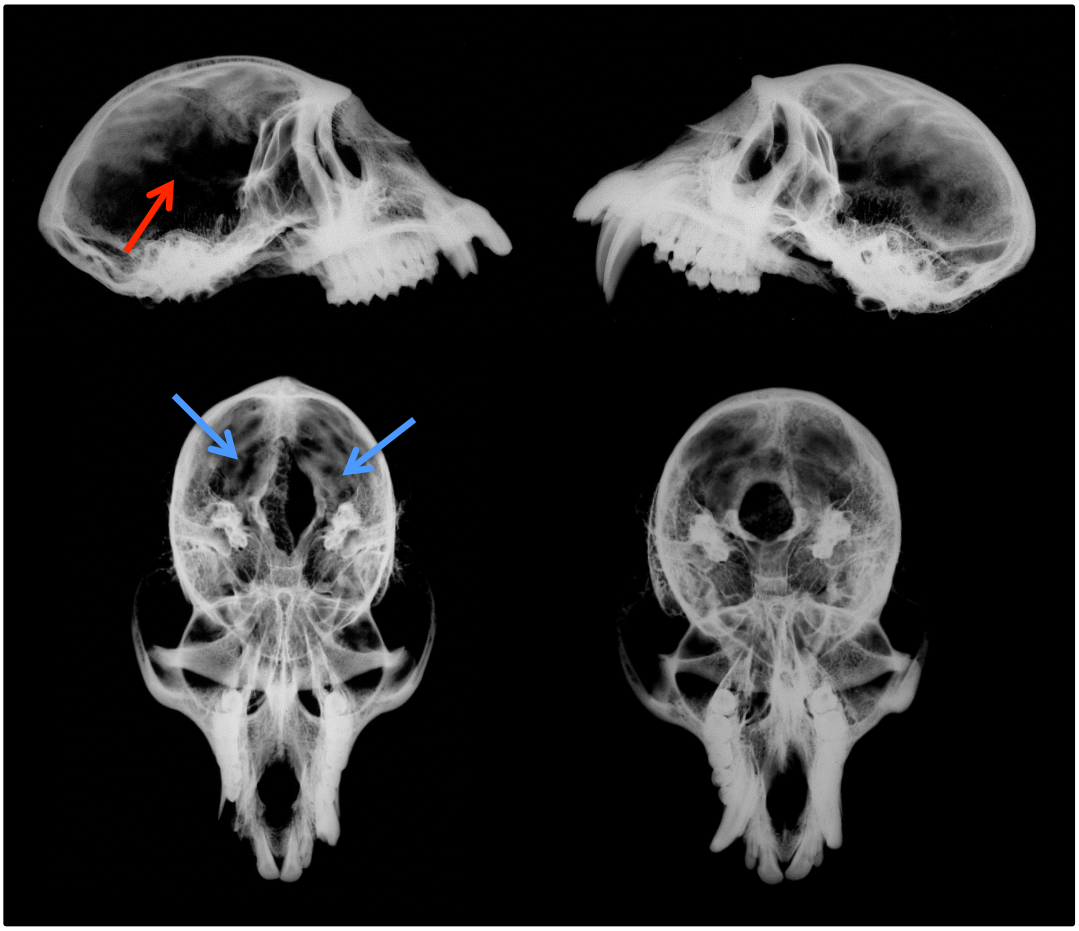

Figure 4.5. Radiographic imaging showing affected (left) and unaffected (right) skulls, seen from a lateral aspect (top) and a dorsal aspect (bottom); where a marked thinning of the cranium is shown by reduced opacity (red arrow) and areas of 'copper-beating' (blue arrows) can be seen in affected skulls from both radiographic imaging and by 'first-hand' observations.

pressure increases to abnormal levels, the cerebrospinal fluid around the brain causes convolutional pitted markings to the inner table of the skull, resembling the copper-beaten effect produced by a ball-peen hammer by coppersmiths. The leading edges of the affected foramen magnum appear sharp and not rounded as is seen in an unaffected skull. Often, the edges show some level of erosion, as too do the occipital condyles. Basilar invagination and associated 'lipping' is not observed. In affected individuals, a distinct crease in the region of the glabella at the superior aspect of the nasal bone is seen in 57.1% of cases and often (35.71%) within this crease is a small, supernumerary accessory bone, measuring a maximum length of 3.6mm by a maximum width of 3.2mm. The nasal ridging typically presents as a series of 3-6 closely-spaced, horizontal ridges, measuring up to 8mm in length.

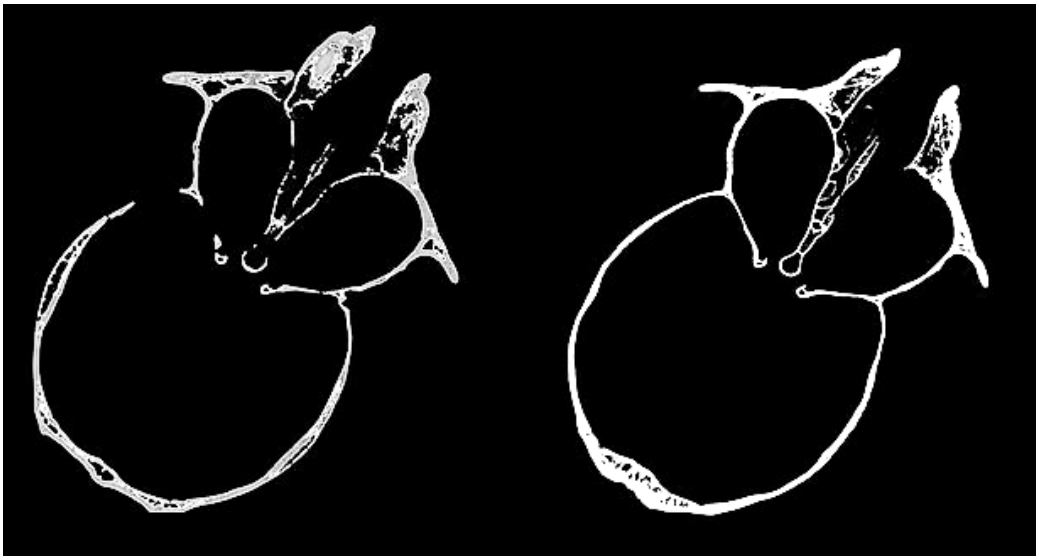

Figure 4.6. Computed Tomography images from an unaffected (left) and pathological (right) individual. Images in the transverse plane show an overall thinning of the skull, both facially (around the zygomatic region) and in the calvarium. Although anecdotal with just two crania imaged, a loss of the diploe is clearly seen in the pathological skull in the mid-parietal region.

4.3 Differential diagnosis

In terms of identifying this pathology, it was important to look at as many relevant potential diseases and conditions as possible. Whilst some pathologies may have initially appeared similar in their aetiology to the Caribbean *Chlorocebus* pathology, either the degree of particular symptoms or the pre-requisite symptoms needed for specific pathologies were not met in these instances.

4.3.1 Sex

Whilst there are many sex-specific pathologies, only a very few (such as Turner's syndrome) affect the skull (Aufderheide and Rodriguez-Martin, 2011; Ros *et al.*2013) and none present symptoms consistent with the *Chlorocebus* pathology described here.

4.3.2 Trauma

Both accidents and population-level disorders can lead to traumatic skeletal pathologies, and many of these can be readily identified by the specific patterns made by the individual traumas. The main category of 'global' cranial trauma is a depressed skull fracture (Bourekas and Lanzieri, 1994), where blunt force causes damage typically characterised by radiating patterns of fractures which originate from the site of impact (Baugnon and Hudgins, 2014). Whilst most fractures occur in either the craniofacial region or within the cranial vault, an estimated 7-16% of non-

penetrating head injuries (in humans) result in fractures extending through the floor of the anterior, middle, or posterior cranial fossa (Baugnon and Hudgins, 2014). Such skull base fractures typically show non-specific patterns of radiating fractures which affect either just the outer table or the outer and inner tables (Schaller *et al.* 2012; Wani *et al.* 2013). These skull fractures occur in individuals as a response to specific accidental trauma and show a non-specific patterning. Neither do they occur in a uniform way at a population level. One of the very few instances where population-level cranial trauma can be seen is in cases of spontaneous depressed skull fractures in new-borns, resulting from complications during traumatic vaginal deliveries (Arifin *et al.* 2013). Whilst this is a highly unlikely scenario in humans and has not been recorded yet in non-human primates, if inbreeding had caused malformations of the female reproductive tract, then such cranial trauma could conceivably be seen at a population level. Myostosis ossificans traumatica is an alternative trauma-originating condition, produced by the avulsion of tendinous/muscular attachment to bone, generating a haematoma. This results in a calcified and typically ossified mass of woven bone (Aufderheide and Rodriguez-Martin, 2011). Whilst cranial traumas such as depressed skull fractures and myostosis ossificans traumatica often show levels of osteolysis associated with the injury (Hećimović *et al.* 1999), the level and type of resorption does not match that of the pathology described here. This, and the non-specific nature of the damage patterning precludes cranial trauma from being the causative agent of the observed pathology.

4.3.4 Circulatory

Whilst looking for the signs of impact from an aneurysm on ‘dry’ bone is relatively difficult, occasionally the impact of an aneurysmal event can be seen in terms of relatively smooth, sharply-demarcated areas of erosion (Aufderheide and Rodriguez-Martin, 2011). This does not match the nature of the symptoms of the observed pathology. Another potential circulatory pathology is thalassemia, a congenital haemolytic anaemia, which being characterised by skull thickening, widening of diploic space, and the involvement of facial bones (Bourekas and Lanzieri, 1994) does not fit the thinning of the skull bones and the loss of diploic space observed in the *Chlorocebus* pathology.

4.3.5 Degenerative

Degenerative joint disease (traditionally called osteoarthritis) is largely a non-inflammatory, chronic, pathological condition characterised by the loss of joint cartilage and subsequent lesions resulting from direct interosseous contact within synovial joints. It is a degenerative condition that may exhibit various degrees of inflammation involving joints and surrounding tissues, potentially leading to anatomical abnormalities and debilitation (Jurmain, 2000; Arzi *et al.* 2013; Roemer *et al.* 2014). In the skull, this disease is only seen at the temporomandibular joint (Kreutziger and Mahan, 1975; Arzi *et al.* 2013). Degenerative joint disease is a major pathology affecting skeletal tissue and is seen at a population level in both humans and animals (Arzi *et al.* 2013). In dry bone, the condition is often

characterised by the presence of desiccation, fragmentation and fissures and at a macroscopic level, the bone matrix can appear thin and fragmented (Bogduk, 2012). Whilst degenerative joint disease has been well-documented in wild non-human primates at a population level (Jurmain 2000; Nakai 2003), it is however associated with synovial joints and results from long-term mechanical force, often with a severe associated inflammatory response of surrounding skeletal tissue (Bogduk, 2012). These factors make the observed *Chlorocebus* pathology described here highly unlikely to be degenerative in nature.

4.3.6 Infectious

Covering both infectious diseases and individual (non-specific) infections, this is a very broad group of potential pathological conditions, comprising of broad bacterial, viral, fungal, and parasitic origins. Osteomyelitis is an inflammation of bone and bone marrow caused by pus-producing bacteria (Aufderheide and Rodriguez-Martin, 2011) and whilst osteomyelitis of the skull is uncommon (Shama, 2012), when it does occur in the skull base, severe bony erosion is observed (Yamazaki *et al.* 2014). Consequently, there is a very high mortality rate in cases of skull base osteomyelitis. Often presenting as a secondary result of diseases such as tuberculosis and syphilis, or as a result of trauma, osteomyelitis often presents with osteopenia, and has a permeative destructive 'moth-eaten' appearance. The most common cause of cranial osteomyelitis is paranasal sinus infection (Bourekas and Lanzieri, 1994).

Yaws is a bacterial infectious disease, closely resembling the causative agent of syphilis (Mitja *et al.* 2013). Yaws disease and syphilis (when in the skull) present with severe traumatic and destructive areas of bone table-penetrating lesions (Meyer *et al.* 2001; Leurero *et al*. 2007). Skeletal tuberculosis (TB) is rare, occurring (in humans) in about 1% of cases of tuberculosis, with presence in the skull only being observed in 0.2-1.3% within this group (Tsui *et al.* 1993; Garcia-Garcia *et al.* 2013). The incidence in non-human animals is thought to be even lower. Radiographic imaging shows a 'punched-out' appearance to the destructive lesions on the bone (Brown *et al.* 1980), with the frontal and parietals being involved more frequently than other areas of the skull. Perforation of the skull is often seen in cranial cases of TB. Often, multiple areas of osteomyelitis are seen in cases of skeletal tuberculosis (Tsui *et al.* 1993). Whilst these (and other) infection-based pathologies can affect cranial tissue and can be present at the population level, their effects are typically highly destructive in appearance and present with a highly irregular appearance, and because of this, the observed *Chlorocebus* pathology is not likely to be infection-based in its aetiology.

4.3.7 Neoplasms

A neoplastic pathology is any mass of localised tissue growth whose cellular proliferation is no longer subject to the effects of normal growth-regulating mechanisms (Aufderheide and Rodriguez-Martin, 2011). Benign tumours arising from the intrinsic vasculature of the bone are the most

common types of cranial neoplasms (Naama *et al.* 2008) and within this category, metastatic disease is the most common neoplastic pathology involving the skull, yet still only accounts for less than 4% of cases of metastases in humans (Khmeleusky and Bychkova, 2015). Here, multiple lytic lesions with somewhat poorly defined margins are typically observed (Bourekas and Lanzieri, 1994). In looking at primary tumours, although they are generally uncommon within the skull, osteomas are the most common neoplasms within the crania of both humans and non-human animals (Haddad *et al.* 1996; Pérez *et al*. 2004; Johnson *et al.* 2014). Developing only in intra-membranous bones, such as the inner or outer tables of the skull and being composed of dense lamellar bone, osteomas present as dense, well-circumscribed, rounded bone-forming lesions (Bourekas and Lanzieri, 1994; Pérez *et al.* 2004), with a central nidus of vascular osteoid tissue surrounded by sclerotic bone (Radcliffe *et al.* 1998). Whilst some bone-affecting neoplasms may have a genetic component which could become prevalent in an insular population (Noterman *et al.* 1997), these, and other rarer bone-affecting tumours (such as hemangiomas and meningioma) do not present standardised symptoms (Estall *et al.* 2010) which would fit with the observed pathology and as a result, the described pathology is unlikely to be of a neoplastic origin.

4.3.8 Idiopathic

There are several idiopathic pathologies, where the aetiology and epidemiology are either unclear or unknown, which affect the skeleton and in particular the skull in both humans and non-human primates (Olson *et al*. 2015). Paget's disease is a common pathology of unknown origin or cause, characterised by bone destruction followed by proliferation of immature woven bone, which is destroyed and reformed repeatedly (Bourekas and Lanzieri, 1994). Paget's disease is usually localised in a single skeletal element or a few bones only and the skull's involvement is seen in 65% of cases. Within these instances, basilar invagination is often seen. A considerable increase in intracranial pressure, which causes thinning of the vault and base of the skull is also commonly encountered (Lane, 1888). Paget's disease is further characterised by gross and disorganised bone remodelling and is frequently accompanied by fracture (Slon *et al.* 2014). Leontiasis ossea is another idiopathic condition and affects only the skeletal tissue of the skull but it is characterised by both distinctive morphological features and the massive deformity of bone (Collier, 1901) and could not be misdiagnosed with any other condition. Once referred to as 'senile atrophy', this is a poorly understood (and unnamed) pathology characterised by severe thinning of the parietal bones (Barnes, 1994), to the extent where the parietal bones actually collapse in patients. None of these idiopathic conditions fit the suite of symptoms associated with the observed *Chlorocebus* pathology.

4.3.9 Metabolic

Vitamin deficiencies and intoxication can, and often do result in various skull changes and deformities. Acute hypervitaminosis A can result in hydrocephalus and bulging of fontanelles in juveniles. Chronic hypervitaminosis A has been associated with poor mineralisation of the skull, with thinning and softening of the bone. Often, dense sutural margins are visible (Bourekas and Lanzieri, 1994). In relation to its stimulation of osteoblast formation (Wendling *et al.* 2009), an excessive intake of vitamin A has been linked to increased risk of fractures (Johansson *et al.* 2002). Hypervitaminosis A is also linked with fragile bones and marked resorption (Katz and Tzagournis, 1972) with joint ankolysing and erosion of articular facets. Vitamin A toxicity in animals can be associated with decreased bone length, thinning of cortical bone and decreased amounts of spongious bone (Slon *et al.* 2014). The condition also commonly presents diffuse idiopathic skeletal hyperostosis (DISH) as an associated pathology (Wendling *et al.* 2009). A deficiency in vitamin D is another commonly seen metabolic pathology which affects skeletal tissue. Resulting from either a lack of exposure to sunlight, inadequate cutaneous biosynthesis, poor dietary intake, or malabsorption (Whyte and Thakker, 2013), rickets is the subsequent clinical pathology due to impaired mineralisation of mixed matrix throughout a growing skeleton. In adults, osteomalacia is the counterpart pathology, after growth plates have at least partially begun to fuse (Whyte and Thakker, 2013). Both rickets and osteomalacia manifest with poor mineralisation, thinning of skull bones, and widening of sutures,

in association with poor sutural mineralisation (Gorlin, 1951; Bourekas and Lanzieri, 1994; Elder and Bishop, 2014; Farrell *et al*. 2015). In hyperparathyroidism, skeletal abnormalities are encountered in approximately 30% of cases, with 50% of these having skull pathologies. The skull typically has a mottled demineralised 'salt-and-pepper' appearance. The condition is often associated with a thickening of the calvarium (Bourekas and Lanzieri, 1994). Metabolic conditions do commonly affect the skull and manifest themselves with several of the symptoms observed in the described *Chlorocebus* pathology, such as a thinning of the cortical bone and the erosion of articular surfaces (as was sometimes seen around the occipital condyles) but they do not match to a level sufficient to fully attribute the observed pathology as metabolic.

4.3.10 Developmental

Although the term 'congenital' implies at least some genetic causative component, some congenital conditions are acquired between fertilisation and birth: because of this, the term 'developmental' is used in this instance. Developmental abnormalities are produced by pathological changes in the normal development experienced during intrauterine life and whilst many of these conditions become apparent at or around the time of birth, many others only present themselves years later. The aetiology of many of these defects is still poorly understood (Aufderheide and Rodriguez-Martin, 2011). Whilst developmental conditions such as spina bifida affect very specific, non-cranial regions of the skeleton (Rab,

2013; Rofail *et al.* 2014), others are more generic and are seen throughout the skeleton, such as osteogenesis imperfecta, which is an inherited condition characterised by brittle bones and numerous subsequent fractures (Imbert *et al.* 2014; Valadares *et al.* 2014).

The term “Chiari malformations” (hereafter termed Chiari’s) represents varying degrees of hindbrain herniation through the foramen magnum (Tubbs *et al.* 2012) and encompasses a range of abnormalities involving the skull, spine, brain and dura (Cai and Oakes, 1997). While Chiari’s can be categorised into Types I – IV according to its severity, tonsillar ectopia is present in all forms, along with underdeveloped posterior cranial (parietal) fossae (McCoy *et al.* 1967; Cai and Oakes, 1997). Basal impressions and cranial assimilation with the atlas are occasionally observed, the primitive derangement of skull bones and basilar skull and craniocervical junction anomalies are observed in approximately 50% of cases (Tubbs *et al.* 2012). Basilar invagination of the foramen magnum, a shortening of the clivus and a foramen magnum that is increased in its dimensions are all typically present (Schady *et al.* 1987; Shuman, 1995). Although still not fully understood, Chiari’s is thought to result from both autosomal recessive and dominant inheritance (Coria *et al*. 1983; Zakeri *et al*. 1995; Catala, 1999), with the Mhox gene thought to be involved in some capacity, as it controls the development and final shape of the occipital and its manipulation in embryonic mice resulted in dysplasia and malformation of the occipitals, Basisphenoid and atlas bones (McLain *et*

al. 1992). Chiari's has been widely reported in King Charles Cavalier spaniels in Europe, Australasia and North America, to a point where a foramen magnum deformity is considered almost standard for the breed (Rusbridge, 1997) and is characterised by muscle weakness, impaired motor coordination, ataxia, neuropathy, hypersensitivity and head-scratching (Rusbridge *et al*. 2006). Chiari's Type I (the most severe variant) is often more evident in adults than in juveniles (Massen and Colonbani, 2005) and there is some evidence that this is due to ontogenetic changes in skull morphology as the animal matures (Cerda-Gonzalez *et al*. 2006).

Craniosynostosis refers to the premature fusion of one or more sutures (of the cranial vault) either in utero, infancy or childhood (Bourekas and Lanzieri, 1994; Kotrivova *et al.* 2007). In typical (unaffected) development, full sutural closure (obliteration) in the cranial vault occurs during adulthood, initially on the endosteal surface and then on the external surfaces (Aufderheide and Rodriguez-Martin, 2011). When a suture fuses prematurely however, growth of the calvarium is limited to a direction parallel to the fused suture, and no growth occurs perpendicular to the affected suture. Compensatory growth is often associated either in the skull base or craniofacial region (Bourekas and Lanzieri, 1994; Saponaro *et al.* 2014) and both compensatory occipitoparietal widening and a heel-shaped frontal region (Wes *et al.* 2014) are typically seen.

4.4 Discussion

The observed *Chlorocebus* cranial pathology does not fully match the symptoms or aetiology of any of the potential conditions covered by the differential diagnosis. It does however fulfil some of the criteria for two of the proposed pathologies, namely Chiari's malformation and hypervitaminosis A. The most diagnostic and symptomatic feature seen in the *Chlorocebus* pathology is the enlarged and characteristically diamond-shaped foramen magnum. It has been observed that foramen magnum-based dysplasia (in terms of distorted shape) is very often associated with developmental pathologies (Barnes, 1994).

Because vitamin A toxicity is metabolic and typically results from environmental (mainly dietary) influences, it could be seen at an incidence as high as that seen in the described *Chlorocebus* pathology (21.4% in the adults). In their natural African habitat, *Chlorocebus* monkeys eat a varied diet of leaves, gums, seeds, grasses, fruit, berries and small vertebrate prey (Harrison, 1984; Fedigan and Fedigan, 1988), although they are are opportunistic feeders and will naturally tailor their diets to incorporate significant amounts of cultivated fruits, vegetables and cereals (Saj *et al.* 2001) when given the opportunity. The Caribbean populations have been given such a possibility and live in environments where crops such as corn, sweet potato, bananas, mangos, papayas, guavas, cherries, cucumbers, peanuts and yams all constitute a significant part of their diets

(Horrocks & Baulu, 1994; Boulton *et al.* 1996; Saj *et al.* 2001). Many of these and other available cultivated crops are rich in high levels of vitamin A. The observed *Chlorocebus* pathology does share several symptoms with hypervitaminosis A; mainly a global thinning of the cranial vault. Vitamin A toxicity also often leads to erosion of articular facets in some affected individuals and a marked resorption of bone. Within the observed *Chlorocebus* pathology, the articulating surfaces of the occipital condyles were in some cases partially (and sometimes severely) eroded and bone resorption was seen in the reshaping of the foramen magnum itself. The observed *Chlorocebus* pathology does not fully fit with hypervitaminosis A however in that vitamin toxicity does not cause a marked increase in intracranial pressure and does not cause remodelling of the foramen magnum specifically.

Chiari's malformation is typically characterised by a distorted and enlarged foramen magnum, with bold and clear lipping, giving it a definite ‘rolled’ appearance. In comparing the shape of the foramen magnum observed in the *Chlorocebus* pathology with Chiari's, the *Chlorocebus* pathology is distinctly diamond-shaped in appearance. Both ends are acutely angled and the leading edges of the foramen magnum do not show basilar invagination. In Chiari’s, the foramen magnum is egg-shaped in appearance, with the proximal aspect being acutely-angled and the distal end being broadly rounded. In most cases, significant basilar invagination is apparent. If the condition seen in the *Chlorocebus* pathology does share

an aetiology with Chiari's, observed differences may stem from basic morphological disparity between human and non-human primate skulls in terms of the positioning of the foramen magnum. However, in other species, Chiari's still manifests in the same way that it does in humans (Rusbridge, 2007). One of the main reasons the observed *Chlorocebus* pathology does not constitute Chiari's is the difference in shape of the foramen magnum and the total lack of basilar invagination. Within the observed *Chlorocebus* pathology, because basilar invagination and an ovoid foramen magnum are not standard symptoms, and given that Chiari's has been medically investigated so extensively and has not been previously described in non-human primates, it cannot be classed as Chiari's malformation, in as far as Chiari's is currently described and defined.

The observed *Chlorocebus* pathology shares symptomatic and aetiological factors with both hypervitaminosis A and Chiari's malformations but importantly, neither condition fully explains the actual pathology. Potentially, the foramen magnum deformity is different in shape when compared to those seen in cases of Chiari's due to differing species-specific morphology of the skull and foramen magnum positioning. Where there was probable elevated intracranial pressure, as shown by the copper-beating effect, such physical differences may potentially serve to release this pressure more evenly, which would result in the lipping typically associated with Chiari's to not be present. Despite the loss of

diploic bone and overall thinning of the skull, this pathology has not been seen to result in either partial or complete penetration within the cranial tables of the cranial vault, meaning that it is not seriously detrimental to the survival of the individual. Because of this, a generalised thinning of the skull could have been passed on and retained at a population level. It is however, important to remember that only two crania were compared in terms of measuring thinness in a quantitative manner. This means that although a cranial thinning is readily seen by an experienced observer, the results referring to thinness are largely anecdotal. The inclusion of further samples should elucidate these findings.

In some instances, a condition which neither affects the structure of the brain nor the development or morphology of the skull e.g. adult hydrocephalus, but which increases intracranial pressure, could produce a copper beating effect, where the increased pressure pushes away at an otherwise-ordinary skull. In this pathology however, the opposite appears to be occurring; normal levels of intracranial pressure acting on a congenitally abnormal (thin) skull is sufficient to mark the inside of the skull, causing the observed copper beating effect. The presence of a small and amorphous supernumerary bone in over 35% of skulls displaying the pathological condition is indicative of an abnormal development of the growing skull.

4.4.1 Founder effect

The founding of a new population on a geographically remote island can provide a window on the colonisation processes unobscured by the smoothing effects of immigration (Baker and Jenkins, 1987). Selective pressures acting on introduced insular populations are likely to be different, because the environment of island ecosystems very often present different characteristics (Planes and Lecaillon, 1998) compared to the original habitat. Although the exact numbers of released monkeys is not known, the original population was likely to have been limited in its size. In such introductions, when a limited number of animals are released, after several generations the majority of individuals within a population will often be fairly closely related, with a subsequent reduction in genetic fitness (Biebach and Keller, 2012; González *et al.* 2005). Any given genetic mutation found in a limited population may become significant during, and subsequent to, colonisation (Planes and Lecaillon, 1998) and such founder effects have led to a high prevalence of several heritable population-level deficiencies, diseases and disorders within some island populations (Lee *et al.* 2009). Founder effects do not always present pathological consequences immediately, as the significant effects of a few initial settlers in the overall population can appear several centuries later (González *et al.* 2005). If an animal in the founding population had a skull which was abnormal in that it was pathologically thin, then this animal may have passed on this condition to subsequent generations and due to either founder effect or genetic drift, the prevalence of a thin skull may have

increased significantly.

With two significant and diagnostic symptoms being present within the observed *Chlorocebus* pathology (a grossly distorted foramen magnum and an overall thinning of the calvarial bones), whether there is a link between the two symptoms should be addressed. Potentially, an underlying genetic condition associated with inbreeding causes both the malformation in the foramen magnum and the thinning of the cranial bones. Alternatively, either the malformation of the foramen magnum causes a thinning of the skull and associated copper beating effect, or a general thinning of the skull bones causes a subsequent distortion of the foramen magnum. The possibility of an underlying inbreeding-associated genetic condition causing the pathology is unlikely, because the original source population for St Kitts is likely to have been large enough and from at least several western African locations to preclude the presence of a pathology of this nature. In dog breeds where a similar cranial deformity is seen, almost the entire global population has been sired by four-five animals seven-eight generations ago, leading to major inbreeding depression within the population. It is also unlikely that a foramen magnum deformity led to subsequent cranial cortical thinning, because in a broad sense, the nature of the foramen magnum distortion effectively makes it larger and would seemingly serve to release an increased pressure within the skull. Whilst it is very difficult to understand the complete aetiology of a pathology based on 'dry bone' alone (Aufderheide and Rodriguez-Martin,

2011), it appears that two separate conditions are occurring in conjunction with one another, to generate the observed *Chlorocebus* pathology. One condition is leading to an increase in intracranial pressure (thus accounting for the copper beating) but is not so severe that it kills affected individuals in utero or in infancy. This first condition appears to have a gradual build-up and an adult onset, explaining why it is not seen in infants or juveniles. The second condition is an overall thinning of the bones of the skull. The presence of two such conditions together may explain how a severe, Chiari's malformation-like pathology is seen without the condition actually being present. To our knowledge, a similar case has not been described in the veterinary or medical literature.

4.5 Conclusion

Of the three Caribbean island populations of *Chlorocebus* monkeys, the observed pathology has only been documented on St Kitts and Nevis. It is proposed here that due to potential founder effect, a genetically predetermined thinning of the skull is seen in the St Kitts population of *Chlorocebus*. The subsequent introduction of monkeys onto Nevis (from St. Kitts) may explain how the pathology has also been seen in Nevis (but at a much lower density) but not at all in Barbados. Alternatively, the Barbados monkeys have all been accessed from a biomedical facility where they have been fed a controlled diet, meaning they are unlikely to display any dietary disorders. The general diet for the wild-living monkeys in the Caribbean is very different to that in Africa, in that generally it is comprised of a higher percentage of cultivated crops, many of which are rich in vitamin A (Sade and Hildreth, 1965; Poirier, 1972; McGuire, 1974; Horrocks 1982; Harrison, 1984; Saj *et al*. 2006). Potentially, a population-level dietary toxicity is present throughout the Caribbean, which when coupled with a thinning of the skull bones (which is seen in only some individuals), may explain how severe, Chiari's malformation-like cranial symptoms are seen without the condition actually being present. The presence of such an underlying condition could have become perpetuated very quickly in a small, localised population which was founded by a limited number of individuals and was cut off from subsequent genetic exchange with the species' original source population. This combination

could explain the high incidence of the pathology. If animals on all three islands have roughly the same diet, which is slightly too high in vitamin A, then the addition of the underlying skull-thinning condition (which is at a higher than expected level due to a founder effect) could result in the presence of the described pathology. Whilst it is very difficult to fully explain the aetiology of a rare or unknown condition based only on 'dry bone' material, this is one possible explanation for the observed *Chlorocebus* pathology. The described *Chlorocebus* pathology is not seen in either juveniles or infants, appearing in adults only. The findings here support the idea that an underlying condition which regulates or stimulates cerebro-spinal fluid may be involved. Because the brain is constantly developing throughout the animal's early life and because copper beating is present, then a sudden adult onset such as this, coupled with the already-thinned skull, could cause the observed pathological symptoms. Because bone is relatively plastic in its nature, even adult skeletal material is able to change if, for example, intracranial pressure suddenly increases.

Often, in zooarchaeological and paleopathological research, dry skeletal material is all that is available. The *Chlorocebus* pathology described here and the methods used provide an example of how macromorphological analysis on skeletal material in order to describe and identify an observed anomaly can be used to gain a better understanding of the pathology and its possible aetiological background.

Chapter 5

General Discussion

5.1 Thesis Summary

Contrary to a long-held and popular belief, the Caribbean *Chlorocebus* monkeys are not all descendants from African green monkeys in the Senegambia region. Instead, each island population represents a broadly diverse community showing that animals had been introduced from not only four separate regions in Africa but more importantly, four distinct species of African *Chlorocebus*. From the mitochondrial (mtDNA) molecular evidence available here, individual monkeys in the Caribbean showed strong genetic affiliations with one of these four different source species populations in Africa. This new understanding of the Caribbean *Chlorocebus* molecular phylogeny has implications not only for their taxonomy but also their wide use in biomedical research. Since their island separation, the skulls of these monkeys have changed. Multivariate analyses using 3D geometric morphometrics have demonstrated here that there has been an overall cranial shape change within the Caribbean population as a whole. Although there appeared to be some observable differences between the African and Caribbean monkeys, this was not supported by statistical testing. Visually, the Caribbean crania (from Nevis, St Kitts and Barbados) show approximately the same level of variance within the community as is seen in any of the African species assessed here. These morphological differences may reflect founder effect and subsequent genetic drift but in light of the broad genetic history of these island communities, it is possible that such changes are reflecting changes in selective pressures encountered within these new ecological

niches, such as intraspecific competition, resource availability and physical characteristics of the three islands (Table 5.1). Similar findings have been seen in other introduced insular primates, reflecting changes associated with changes in longitude (Cardini *et al*. 2007) and rainfall (Lehhman *et al*. 2005) and island area (Lomolino, 2005). Future studies that recorded inter-island differences at a local level would permit such potential associations to be assessed for.

	Barbados	**Nevis**	**St Kitts**
Area	431 km^2	93 km^2	176 km^2
Highest elevation	336 m	985 m	1,156 m
Annual precipitation	1000 - 2300 mm	1250 - 2000 mm	700 - 1200 mm
Climate	Trop. monsoon	Trop. monsoon	Trop. Monsoon
Arable land use	34.8%	19.2%	19.2%
Latitude	13^o 19' N	17^o 16' N	17^o 36' N
Longitude	59^o 54' W	62^o 56' W	62^o 56' W

Table 5.1. Showing some of the main physical and climatic characteristics of the three Caribbean islands supporting Chlorocebus monkey populations. Data from FAO, 2016.

As an interesting additional finding, a population level cranial pathology was observed in two of the three island populations. As this severe pathology is not seen in African monkeys and has not been recorded before, it may well represent a new type of cranial malformation. This thesis has identified and investigated a rare example of a complex but well-documented insular introduction event for primates. With known dates of introductions and being spread across three geographically distinct populations, the Caribbean

Chlorocebus monkeys represent an opportunity to thoroughly explore insular primate evolution, ecology and behaviour.

5.2 African *Chlorocebus* monkeys

Although the main focus of this thesis was the three introduced Caribbean populations of *Chlorocebus* monkeys, it was of central importance to first further understand the African *Chlorocebus* taxon from which the Caribbean populations originated. The *Chlorocebus* taxon has an incredibly broad distribution in sub-Saharan Africa, where different species are often contiguous with one another and hybridisation is possible. The African phylogeny is both contentious and still largely unresolved. However, recent mtDNA analyses of African *Chlorocebus* monkeys showed nine major mtDNA clades reflecting geographic distribution rather than taxa (Haus *et al*. 2013), implying that the mtDNA diversity of African *Chlorocebus* does not actually conform to any of the existing taxonomic classifications. The mtDNA phylogeny presented here does not fully support the traditional taxonomy of African *Chlorocebus* designations but instead largely agrees with these more recent mtDNA findings, reflecting the need for a better understanding of the *Chlorocebus* clade as a whole, based on actual geographical distribution rather than nominal species designation. Whilst the data from this thesis are from samples with a limited number of base pairs, the results still strongly support these other recent mtDNA analyses where the whole cytochrome *b* gene was studied.

There has been extensive study into primate cranial morphology, with a tendency to focus on cranial modules such as the olfactory region or the basicranium and not the skull as a whole (Fleagle *et al*. 2010; Gilbert, 2010). This thesis instead focused upon the skull as a whole unit, combining all major cranial anatomical modules. In terms of African *Chlorocebus* cranial shape variance, the use of 3D geometric morphometrics and Principal Components Analysis (PCA) analysis demonstrated there are no clear patterns of division in the three African species of *Chlorocebus* studied here (*C. sabaeus*, *C. tantalus* and *C. cynosuros*) that represent possible source populations for the Caribbean monkeys but instead these species show a slight clinal pattern of variation. Whilst the African cercopithecines are a highly speciose group subject to considerable ecological and behavioural variability, their skulls are much less diverse (Cardini and Elton, 2007a; Cardini and Elton, 2008), and are noted for their general uniformity and lack of diagnostic features. This generalised uniformity in skull shape is evident in the three recognised western species of *Chlorocebus*, showing little if any quantifiable cranial shape difference between species or across a latitudinal scale and that cranial morphometrics are not a reliable way in understanding their phylogeny in any definitive detail.

Despite the inclusion of genetic data from locations not included in previous studies and the use of detailed landmark-based multivariate morphological analyses, the results presented in this thesis are not in agreement with either

of the traditional suggestions but are concordant with more recent data in finding that not only is the African *Chlorocebus* taxon highly complex, it is still unresolved. In terms of the African *Chlorocebus* groupings, no taxonomic changes are considered here at this time.

5.3 Caribbean *Chlorocebus* monkeys

The traditional notion that Caribbean *Chlorocebus* monkeys originate from a Senegal-based *Chlorocebus sabaeus* ancestor (Sade and Hildrech, 1965; van der Kuyl *et al*. 1996) has been disproved within this thesis by looking more closely at the genetics of the introduced populations of *Chlorocebus* with the Caribbean. These findings show that wild-living Caribbean *Chlorocebus* monkeys stem from several western African sources, originating from four different species of African *Chlorocebus* monkeys and not just *C. sabaeus*. The establishment of the Caribbean *Chlorocebus* is not likely to have come from a single colonisation event associated with the trans-Atlantic slave trade but instead represents numerous introductions from multiple sources across an extended period of time.

The cranial morphological data from this thesis represent both the first time all three populations of Caribbean *Chlorocebus* have been studied together and the first time any of the Caribbean skulls have been assessed through the use of 3D geometric morphometrics, giving a more detailed understanding than previous uni- and multivariate analyses on the group. These methods are

more powerful than traditional morphometrics in distinguishing among shape differences after translating specimen configurations to a common origin (Harvati *et al*. 2004; Lockwood *et al*. 2004; Adams *et al*. 2011), scaling them to unit centroid size and rotating them to best fit by using a least square criterion. Primates have been shown to be subject to size changes once introduced to insular environments (Bromham and Cardillo, 2007; Schillaci *et al*. 2009; Welch, 2009; Gardner and Jasper, 2015) but very little research has been done into quantifiable shape differences in these island primates. In looking at the Caribbean *Chlorocebus* populations, although there was some obvious overlap, PCA analyses showed that the skulls from the three island populations were noticeably distinct from one another. There were signs of overlap between the skulls from the St Kitts and Nevis populations which is to be expected, as extensive historical records detail that African monkeys originally introduced onto St Kitts were subsequently introduced onto the neighbouring Nevis over one hundred years later. These results appear to be in contrast with the Barbados samples that sit separately, away from the other Caribbean populations. The Barbados monkeys were introduced in isolation to the other two Caribbean populations and in effect have been left to their own devices since their original period of introduction. Whereas changes in size in primate anatomy appear to be the 'line of least evolutionary resistance' (Marriog and Cheverud, 2005), changes in shape appear to be more conservative, especially in rapidly changing environments (Elton *et al*. 2010). In consideration of this, it would appear that something has driven cranial shape change in the Caribbean *Chlorocebus* monkeys, especially within the

Barbados population. Although not supported statistically, it appears that male and female samples sit separated from one another, displaying the sexual dimorphism typical of cercopithecine monkeys (Cardini and Elton, 2008). Between the three Caribbean populations, females showed clearer distinction from one another compared to the males. There was no overlap between the female samples across the island populations (Fig. 3.7.1). Whilst it is possible these apparent shape differences across the Caribbean populations are due to founder effect and subsequent genetic drift or the effects of a low sample size, the effects of strong intraspecific competition for limited resources, harsh environmental conditions and heavy mortality typically experienced by introduced island populations (Wright, 1999; Lomolino, 2005; Woolfit and Bromham, 2005) may have driven alterations in cranial shape changes as a result of changes in the ecology and behaviours experienced by these island primates.

Within the Caribbean islands populations of *Chlorocebus* monkeys, an observed cranial pathology has been documented on St Kitts and Nevis, most likely resulting from a genetically predetermined thinning of the skull due to founder effect. Additionally, the general diet for the Caribbean monkeys is very different to that in Africa, in that generally it is comprised of a higher percentage of cultivated crops, many of which are rich in vitamin A (Sade and Hildreth, 1965; Poirier, 1972; Horrocks 1982; Harrison, 1984; Saj *et al*. 2006). Potentially, a population-based low-level vitamin dietary toxicity is present throughout the Caribbean which, when coupled with the thinning of the skull

bones seen in some individuals, may explain how the symptoms of an already well-known aetiologically-complex and typically lethal human cranial malformation are seen without the condition actually being present. The presence of an underlying condition such as a thinning of the cranial bones could have become perpetuated very quickly in a small, localised population which was founded by a limited number of individuals and was cut off from subsequent genetic exchange with the species' original source population. If animals on all three islands have roughly the same diet which is slightly too high in vitamin A, then the addition of the underlying skull-thinning condition on two islands could result in the presence of the observed pathology. Whilst it is very difficult to fully explain the aetiology of a rare or unknown condition based only on 'dry bone' material, this is one possible explanation for the observed *Chlorocebus* pathology.

In looking at the Caribbean *Chlorocebus* populations, whilst the molecular phylogenies are not fully resolved, this study has for the first time revealed that these island communities are comprised of animals from numerous and distinct African lineages, from along much of the western coast of Africa. These clearly distinct phylogeographic lineages within the individual island populations are possibly being maintained through the use of facial-based character displacement visual signals, acting as isolating barriers to reduce gene flow between heterospecific phenotypes. The results presented here show it is no longer accurate to refer to these Caribbean animals simply as 'green monkeys' or even African green monkeys, as they represent animals

from several African species. Additionally, cranial shape across the three Caribbean populations has been shown to respond to being isolated in insular conditions. The skulls between the three islands are broadly distinct from one another, with similarities between St Kitts and Nevis skulls reflecting a more recent split than those from Barbados, which are morphologically distinct. The presence of a significant cranial pathology found at a population level on two of the three island communities represents an example of how when dry skeletal material is all that is available (as is often the case with zooarchaeological and paleopathological research) the macromorphological analysis of an observed anomaly on skeletal material can be used to gain a better understanding of the pathology and its possible aetiological background.

5.4 Comparing African and Caribbean *Chlorocebus* monkeys

Despite the potentially limited nature of the molecular samples within this study, the results were still rigorous enough to support the most thorough existing genetic analyses conducted on the *Chlorocebus* taxon (Haus *et al*. 2013). Given the potential of even limited samples generating results concordant with genome-wide analyses, there were no discernable changes between the mtDNA genetic profile of Caribbean individuals and their African counterparts. While it appears that the Caribbean populations each originated from a mixture of African species, no population-level changes have been seen at this time.

Previous cranial analyses focused on size differences between the St Kitts Caribbean population and West African population of *C. sabaeus* (Ashton and Zuckerman, 1951a; Ashton and Zuckerman, 1951b; Ashton *et al*. 1997) and found that the St Kitts skulls were bigger and less variable. In looking at all three of the Caribbean populations of *Chlorocebus* here for the first time, although there were some obvious areas of overlap, Principal Components Analysis (PCA) showed that in terms of shape, the skulls from the three island populations not only appeared different from one another but were discernable from the African species' crania. This finding however was not statistically supported. The male skulls from St Kitts and Nevis were similar to one another and did show some overlap with African *C. sabaeus* skulls but were still identifiably distinct. In contrast to this, male skulls from the Barbados population were wholly distinct from any of the African populations, demonstrating that this community has undergone significant changes in cranial shape since their separation from Africa just 400 years ago. Similarly, when comparing female skulls from the three Caribbean populations with those from African *C. sabaeus* crania, populations from the Caribbean all appeared distinctly different from the African skulls in terms of their overall shape. From the mtDNA molecular analyses, it is apparent that Caribbean *Chlorocebus* monkeys share a close mtDNA relationship with at least three distinct African *Chlorocebus* species, therefore highlighting the conclusion that the Caribbean populations have a broadly mixed molecular heritage and must have originated from at least three distinct introduction events, from across

three African source species. The majority of Caribbean *Chlorocebus* monkeys are directly descended from African *C. sabaeus* monkeys from the Senegambia and Sierra Leone region of western Africa. Monkeys from Barbados and St Kitts show this clear affiliation, with further close relationships with those originating from Ghana and Burkina Faso. These findings support the existing view that Caribbean *Chlorocebus* monkeys are descended from African *C. sabaeus* animals. However, the findings here have revealed an additional facet to this long-held idea in that some Caribbean samples originated from *C. tantalus* animals from around Nigeria, while others (from both St Kitts and Barbados) are most closely related to *C. cynosuros* samples from the area around Zimbabwe and its neighbouring countries. This analysis clearly shows that the monkeys introduced into the Caribbean were taken from distinctly different species of African *Chlorocebus* from distinctly different locations from across coastal western Africa. This finding is supported by the presence of significant ports of disembarkation from across the western African seaboard, which fed the trade in enslaved Africans (Fig 1.2). With discrepancies such as this and an apparent level of ambiguity with the majority of the samples originating from Ethiopia, the African *Chlorocebus* taxonomy is in need of revision. A strong (highly supported) level of distinctiveness (in terms of shape) was observed between the three Caribbean populations. When compared to the mainland African *Chlorocebus*, the Caribbean populations showed a higher level of distinction (between islands) than was seen between African species.

The question of exactly ‘what is a species’ is of fundamental importance within evolutionary biology, ethology and conservation management and has been debated and argued over for decades. Regardless as to whether this question can ever be fully resolved, ‘how does a species arise’ is down to mechanisms, which maybe genotypic, phenotypic or environmental (or some combination of the three). Of central importance is the concept that through some such regulatory mechanism, a single, relatively homogenous group or population at some point is the founder of two (or more) distinct subsequent groups or populations and that these ‘new’ populations can no longer exchange their genetic material (either with each other or with their founding population). The example of *Chlorocebus* monkeys being transported from mainland Africa to three islands within the Caribbean has clearly fulfilled the criteria (at least in theory) necessary for a new species to begin its ascent; as the Atlantic Ocean now acts as a very apparent reproductively isolating barrier, preventing any contact between the Caribbean populations and their mainland African founders. Despite there being six described (and largely accepted) species of African *Chlorocebus* monkeys, for the most part they are not wholly reproductively isolated to the point where interbreeding is untenable and hybridization has been observed where species sit alongside one another. Ironically, the introduced Caribbean *Chlorocebus* monkeys now represent the only member of the *Chlorocebus* genus (and one of the very few cercopithecines) to be wholly reproductively isolated. Additionally, islands are renowned for their effects on both accelerating evolutionary change and causing often-exaggerated adaptations. These responses to insular

environments are as a result of a range of influences; from abiotic factors such as levels of rainfall, land mass and island elevation, to biotic factors including reduced predation, increased intraspecific competition and a change in the availability (and type) of food. Often, islands are sub-optimal habitats and can cause significant developmental stress on introduced populations and this too can be responsible for observable change in 'new' island populations. Increased bilateral asymmetry and cranial shape change have both been associated with a rise in developmental stress and both have been observed within the Caribbean *Chlorocebus* populations, with the Caribbean population crania showing greater variation that between African species. With the Biological Species Concept still being the most widely-used and accepted means to define a species, it can be said to be operating on different *Chlorocebus* groups in different ways. To fulfill this concept, the populations need to be reproductively isolated (from one another). Whereas the presence of significant geographical barriers and physical adaptations such as hair and facial coloration do serve to largely isolate the six African species, these boundaries are seemingly not insurmountable and clinal change is seen across the African *Chlorocebus*. The Caribbean monkeys however are entirely isolated, not only from their African ancestors but also from each other. Under the Biological Species Concept (and given adequate time), the Caribbean monkeys may one day warrant the designation of a new species. However, other species concepts may entail a different outcome. Whereas the Typological (or Morphological) Species Concept may separate a new population such as these Caribbean monkeys if they are morphologically

distinct, it largely depends on readily observable (usually external) differences, such as hair and facial coloration and these have not yet been quantified within the Caribbean monkeys. From the perspective of the Phylogenetic Species Concept however, the Caribbean monkeys would remain under the umbrella of the African *Chlorocebus*, which (with a shared recent ancestry) would themselves be a monophyletic group. The example of a medium-sized, widespread mammal such as the African *Chlorocebus* monkey being introduced to a novel insular ecosystem provides not only a very tangible point from which to further explore species concepts but also represents a potentially important opportunity to quantifiably assess the early steps of speciation.

5.5 Suggestions for future work

Interestingly, there were no discernable changes between the mtDNA genetic profile of Caribbean individuals and their African counterparts. Such a lack of strong significant differentiation between island populations and across African species is consistent with the recent history of the Caribbean monkey introductions and the insensitivity of mtDNA to small-scale or recent population events (Kirschning *et al*. 2007; Ackermann *et al*. 2014; Langille *et al*. 2014). The use of mtDNA has several advantages including: the lack of recombination, a rapid coalescence time, relatively high substitution rates, high copy number, and haploidy. However, such methods are ideal for obtaining and sequencing data in low quality samples such as museum specimens (Rowe *et al*. 2011; Pozzi *et al*. 2014), which were used heavily in this study. While the samples were relatively small and used only a limited number of base pairs, the results were still congruent with the most recent molecular data that focused on the African *Chlorocebus* taxon (Haus *et al*. 2013). This is likely to mean that the lack of clear differences between the African and Caribbean monkeys is either explainable either due to any existing differences not yet being apparent in the well-conserved mtDNA profile or because any such differences are so small the complete Caribbean mtDNA genome would need to be assessed, or because any differences would be better explored using nuclear DNA (nDNA). Given that differences have now been observed between the Caribbean and African *Chlorocebus* and that the results here tantalisingly hint at an unexpected and potentially

complicated series of island introductions, the use of nDNA for further investigation would enable any changes to be explored and the development of a better understanding of primate island biogeography. In considering that the African *Chlorocebus* taxon is still unresolved and largely contested, then the use of nDNA analysis on what represents the most widely-spread taxon of African primates would permit a fuller understanding of the whole group and a wider understanding of primate biogeography in general.

Within the Caribbean, *Chlorocebus* monkeys are widely used by biomedical laboratories to investigate and develop HIV/AIDS research through looking at viral replication and immune responses in natural hosts infected with SIV (van der Kuyl *et al*. 1996; Pandrea *et al*. 2006), with the research being based on the assumption (from a limited dataset) that all Caribbean *Chlorocebus* monkeys are *Chlorocebus sabaeus.* Each *Chlorocebus* species has its own distinct SIV subtype in the wild, yet laboratory animals are all infected with the *C. sabaeus*-specific SIVagm.sab subtype and are then monitored and treated according to this precept. In light of the results presented in this thesis and the real threat for inaccurate results from such wide-reaching biomedical research, further investigation is needed to wholly resolve both the taxonomy and phylogeny of Caribbean *Chlorocebus* monkeys, using nDNA analyses.

Further morphological studies that focus on within-archipelago patterns in size and shape variation of a particular taxon such as the Caribbean *Chlorocebus* may provide especially important clues to the wider factors influencing

evolution of biotic factors (Lomolino, 2005; Cardini *et al*. 2007). The results in this thesis show that even after just approximately 400 years, the skulls of introduced Caribbean *Chlorocebus* monkeys have undergone changes in overall shape. Further study into the exact nature of this shape change may elucidate what these influencing factors are and how the Caribbean monkeys have started to functionally adapt to suit these new insular environments. The use of much larger sample sizes, the inclusion of clearly-defined age groups and the use of additional methods such as canonical correlation analysis and partial least squares analysis would allow further investigation into any potential size changes, developmental shape and size differences across age groups and correlating any island population changes with a variety of possible environmental variables such as rainfall, temperature, latitude or diet. Additionally, African samples with complete data detailing the exact origin of the samples would allow further investigation into possible impacts of hybridisation between the African species upon the results (Cardini and Elton, 2007b). The ecological relationships between African and Caribbean *Chlorocebus* monkeys requires more study using a wider source of methodological and analytical techniques. For example, research into the behavioural ecology of the three Caribbean populations; covering their population dynamics, feeding ecology and intraspecific competition, would allow direct comparisons to be made with African *Chlorocebus*. Additionally, building on the methods used in this thesis would permit a more detailed understanding of the whole *Chlorocebus* taxon to be created. Linking morphological and molecular data would provide an invaluable (and more

comprehensive) understanding into any potential link between the phenotypes and genotypes seen across African and Caribbean Chlorocebus monkeys. This was not possible within this study due to many of the molecular samples being retrieved from Genbank and not correlating with morphological scans. Many of the samples where both molecular and scan data are available are for Caribbean samples and without a wider range of African samples to complement, little would be revealed by such analysis here. Future work to collect African samples (where both African and Caribbean samples are available) would permit a comprehensive analysis to be made to explore potential links between phenotypes and genotypes across the entire *Chlorocebus* distribution. These additional areas of study and more detailed techniques should be applied to this unique and fascinating case study, where the very early stages of speciation in several introduced populations of primates from the same taxon can be studied under well-documented insular 'natural laboratory' conditions.

5.6 Conclusions

This study represents a broad experimental approach to investigate the origin and development of a series of introduced primate island communities. Results here have for the first time conclusively shown that the Caribbean *Chlorocebus* monkeys are not simply introduced *Chlorocebus sabaeus* from the Senegambia region in western Africa. Instead, these Caribbean monkeys live in large populations of broadly mixed origins, from four different African species of *Chlorocebus.* Additionally, the overall cranial shape of Caribbean *Chlorocebus* monkeys has changed since their introduction. This shape change is discernable not only between African and Caribbean crania but also between the crania from the three Caribbean island populations. The morphological differences between the Caribbean *Chlorocebus* crania were more statistically distinct from each other (between island populations) than the nominal species on mainland Africa are from each other.

African *Chlorocebus* monkeys represent an overwhelmingly important group which despite being ecologically interesting and phylogenetically complex group, had for a long time received less than the attention they deserved. The introduced Caribbean *Chlorocebus* monkeys fared worse and were written off as simply being a convenient offshoot from one of these African groups and were seen to have little if any significance to the research community, despite being used for biomedical research. The findings within this thesis may not only have an impact on such biomedical research where it appears the

primates have alarmingly been incorrectly identified, possibly confounding subsequent findings but also represents an important contribution in exploring and understanding the very early stages of speciation in insular primates. Combining these findings with further behavioural and ecological research could help elucidate the driving factors behind the very first steps that may eventually lead to speciation in island-living primates, may further our broader understanding of insular mammalian phylogenies and could have important impacts for the development of conservation management plans for island species around the world.

This thesis has various applications across both anthropological research and conservation management. Cranial anatomical features play a prominent part in the definition of both extinct and extant human, hominid and other primate species (Albessard *et al*. 2016) and an understanding of such morphology can reveal much about a species' evolution, ecology, migration and speciation. That, combined with research on the far-reaching impacts of even small genetic differences between primate species (Scally *et al*. 2012) allows a better understanding within the study of biological anthropology than ever before. The interplay between morphological and molecular data allows us to more comprehensively explore the early stages of speciation and to elucidate the mechanisms that affect introduced insular populations. Further than being solely an interesting opportunity within which insular populations reveal evolutionary processes, they are instead at the very forefront of conservation management. With introduced 'invasives' having a huge global impact on

otherwise ecologically-naïve island species and through mainland habitat loss creating isolated island-like habitats in forest fragments and mountain ecosystems, a fuller understanding of insular species and introduced populations has broad applications across numerous vertebrate and invertebrate taxa and throughout various ecosystems and habitats. Not only are human activities driving species extinctions but also speciation (Bull and Maron, 2016). This study can be viewed as contributing to a more comprehensive understanding of the global biosphere, where human-induced speciation in addition to extinction is changing both flora and fauna.

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Appendix

Appendix 1 Supplementary Information for samples used in Chapter 2

Code	Accession No.	Country Origin	Collection Source	Source	Sequence Length (BP)	Successful extraction
BG001	A74.21	Zimbabwe	*C. pygerythrus*	RCS	259	YES
BG002	A74.22	Zimbabwe	*C. pygerythrus*	RCS	259	YES
BG003	A72.1	Nigeria	Caribbean	RCS	259	NO
BG004	A72.2	Nigeria	Caribbean	RCS	259	NO
BG005	G99.2	Nigeria	*C. tantalus*	RCS	259	YES
BG006	A71.3	Nigeria	*C. tantalus*	RCS	259	YES
BG007	A71.4	Cameroon	*C. tantalus*	RCS	259	YES
BG008	A72.6695	St. Kitts	Caribbean	RCS	259	YES
BG009	A72.681	St. Kitts	Caribbean	RCS	259	YES
BG010	A72.652	St. Kitts	Caribbean	RCS	259	YES
BG011	A72.6696	St. Kitts	Caribbean	RCS	259	YES
BG012	A74.23	Zimbabwe	*C. pygerythrus*	RCS	259	YES
BG013	A72.634	St. Kitts	Caribbean	RCS	259	YES
BG014	A72.618	St. Kitts	Caribbean	RCS	259	YES
BG015	A72.6692	St. Kitts	Caribbean	RCS	259	YES
BG016	A72.635	St. Kitts	Caribbean	RCS	259	YES
BG017	A74.26	Zimbabwe	*C. pygerythrus*	RCS	259	YES
BG018	A72.662	St. Kitts	Caribbean	RCS	259	YES
BG019	A72.8	Ethiopia	*C. pygerythrus*	RCS	259	YES
BG020	A72.14	S. Leone	*C. sabaeus*	RCS	259	YES
BG021	A72.6711	St. Kitts	Caribbean	RCS	259	YES
BG022	A72.646	St. Kitts	Caribbean	RCS	259	YES
BG023	A72.12	St. Kitts	Caribbean	RCS	259	YES
BG024	A74.25	St. Kitts	Caribbean	RCS	259	YES
BG025	A72.6694	St. Kitts	Caribbean	RCS	259	YES
BG026	A72.682	Zimbabwe	*C. pygerythrus*	RCS	259	YES
BG027	A72.648	St. Kitts	Caribbean	RCS	259	YES
BG028	A72.638	St. Kitts	Caribbean	RCS	259	YES
BG029	A74.2	Zimbabwe	*C. pygerythrus*	RCS	259	YES
BG030	A72.633	St. Kitts	Caribbean	RCS	259	YES
BG031	ZD26.11.1.19	DRCongo	*C. cynosuros*	RCS	259	NO
BG032	ZD26.11.1.12	DRCongo	*C. cynosuros*	NHM	259	YES
BG033	ZD24.8.6.7	Nigeria	*C. tantalus*	NHM	259	YES
BG034	ZD7.7.8.3	Nigeria	*C. tantalus*	NHM	259	YES
BG035	ZD48.455	Cameroon	*C. tantalus*	NHM	259	YES
BG036	ZD69.1152	Cameroon	*C. tantalus*	NHM	259	YES
BG037	ZD54.922	S. Leone	*C. sabaeus*	NHM	259	YES
BG038	G53.2	S. Leone	*C. sabaeus*	NHM	259	YES
BG039	A72.6710	St. Kitts	Caribbean	NHM	259	YES
BG040	A72.663	St. Kitts	Caribbean	NHM	259	YES
BG041	ZD1909.11.2.2	Senegal	*C. sabaeus*	NHM	259	YES
BG042	ZD1946.34	Gambia	*C. sabaeus*	NHM	259	NO
BG043	ZD1982.631	Gambia	*C. sabaeus*	NHM	259	NO
BG044	ZD1982.625	Gambia	*C. sabaeus*	NHM	259	NO
BG045	ZD82.629	Gambia	*C. sabaeus*	NHM	259	NO
BG046	ZD1981.1235	Gambia	*C. sabaeus*	NHM	259	NO
BG047	ZD920.7.10.13	S. Leone	*C. sabaeus*	NHM	259	NO
BG048	ZD7.10.3	S. Leone	*C. sabaeus*	NHM	259	NO

BG049	ZD1956.264	Ghana	*C. sabaeus*	NHM	259	NO
BG050	ZD1956.265	Ghana	*C. sabaeus*	NHM	259	NO
BG051	ZD11.6.10.3	Fr. Guinea	*C. sabaeus*	NHM	259	NO
BG052	Nev0901	Nevis	Caribbean	FIELD	259	NO
BG053	Nev0902	Nevis	Caribbean	FIELD	259	NO
BG054	Nev0903	Nevis	Caribbean	FIELD	259	NO
BG055	Nev0904	Nevis	Caribbean	FIELD	259	NO
BG056	Nev0905	Nevis	Caribbean	FIELD	259	NO
BG057	Nev0906	Nevis	Caribbean	FIELD	259	NO
BG058	Nev0907	Nevis	Caribbean	FIELD	259	NO
BG059	Barb0908	Barbados	Caribbean	FIELD	259	YES
BG060	Barb0909	Barbados	Caribbean	FIELD	259	YES
BG061	Barb0910	Barbados	Caribbean	FIELD	259	YES
BG062	Barb0911	Barbados	Caribbean	FIELD	259	YES
BG063	Barb0912	Barbados	Caribbean	FIELD	259	YES
BG064	Barb0913	Barbados	Caribbean	FIELD	259	YES
BG065	Barb0914	Barbados	Caribbean	FIELD	259	YES
BG066	Barb0915	Barbados	Caribbean	FIELD	259	YES
BG067	Barb0916	Barbados	Caribbean	FIELD	259	YES
BG068	Barb0917	Barbados	Caribbean	FIELD	259	YES
*1	JX983734	Ethiopia	*C. aethiops*	Genbank	1,140	YES
*2	JX983735	Ethiopia	*C. djamdjam.*	Genbank	1,140	YES
*3	JX983796	S. Africa	*C. pygerythrus*	Genbank	1,140	YES
*4	JX983732	Nigeria	*C. tantalus*	Genbank	1,140	YES
*5	JX983736	Angola	*C. cynosuros*	Genbank	1,140	YES
*6	JX983733	Ethiopia	*C. pygerythrus*	Genbank	1,140	YES
*7	EF597500	Tanzania	*C. pygerythrus*	Genbank	16,343	YES
*8	EF597502	Cameroon	*C. tantalus*	Genbank	16,368	YES
*9	JX983738	Cameroon	*C. tantalus*	Genbank	1,140	YES
*10	EF597501	Kenya	*C. pygerythrus*	Genbank	16,438	YES
*11	JX983730	Ethiopia	*C. aethiops*	Genbank	1,140	YES
*12	JX983806	Ghana	*C. sabaeus*	Genbank	1,140	YES
*13	JX983836	Burk. Faso	*C. sabaeus*	Genbank	1,140	YES
*14	JX983731	Ethiopia	*C. aethiops*	Genbank	1,140	YES
*15	JX983732	Ethiopia	*C. pygerythrus*	Genbank	1,140	YES
*16	JX983737	Ethiopia	*C. aethiops*	Genbank	1,140	YES
*17	JX983804	Ghana	*C. sabaeus*	Genbank	1,140	YES
*18	JX983837	Burk. Faso	*C. sabaeus*	Genbank	1,140	YES
*19	JX983805	Ghana	*C. sabaeus*	Genbank	1,140	YES
*20	JX983756	Zambia	*C. cynosuros*	Genbank	1,140	YES
*21	JX983843	Nigeria	*C. tantalus*	Genbank	1,140	YES
*22	JX983739	Ethiopia	*C. djamdjam.*	Genbank	1,140	YES
*23	JX983846	CAR	*C. tantalus*	Genbank	1,140	YES
*24	JX983740	Ethiopia	*C. djamdjam.*	Genbank	1,140	YES